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RESEARCH

Thr92Ala-DIO2 heterozygosity is associated with skeletal muscle mass and myosteatosis in patients with COVID-19

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Abstract

Introduction: The type 2 deiodinase and its Thr92Ala-DIO2 polymorphism have been linked to clinical outcomes in acute lung injury and coronavirus disease 2019 (COVID-19).

Objective: The objective was to identify a potential association between Thr92Ala-DIO2 polymorphism and body composition (appendicular muscle mass, myosteatosis, and fat distribution) and to determine whether they reflect the severity or mortality associated with the disease.

Methods: In this prospective cohort study (June–August 2020), 181 patients hospitalized with moderate-to-severe COVID-19 underwent a non-contrast-enhanced computed tomography (CT) of the thorax to assess body composition, laboratory tests, and genotyping for the Thr92Ala-DIO2 polymorphism.

Results: In total, 181 consecutive patients were stratified into three subgroups according to the genotype: Thr/Thr (n = 64), Thr/Ala (n = 96), and Ala/Ala (n = 21). The prevalence of low muscle area (MA) (< 92 cm^2) was 52.5%. Low MA was less frequent in Ala/Thr patients (44.8%) than in Thr/Thr (60.9%) or Ala/Ala patients (61.9%) (P = 0.027). Multivariate logistic regression analysis confirmed that the Thr/Ala allele was associated with a reduced risk of low MA (41% to 69%) and myosteatosis (62% to 72%) compared with Thr/Thr + Ala/Ala (overdominant model). Kaplan–Meier curves

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showed that patients with low muscle mass and homozygosity had lower survival rates than the other groups. Notably, the heterozygotes with MA ≥92 cm^2 exhibited the best survival rate.

Conclusion: Thr92Ala-DIO2 heterozygosity is associated with increased skeletal MA and less myosteatosis in patients with COVID-19. The protective effect of Thr92Ala-DIO2 heterozygosity on COVID-19 mortality is restricted to patients with reduced MA.

Keywords: COVID-19; muscle; myosteatosis; Thr92Ala-DIO2

Introduction

Over the last 3 years, there have been significant morbidity and mortality worldwide caused by the coronavirus disease 2019 (COVID-19), a highly infectious condition caused by the severe acute respiratory syndrome virus 2 (SARS-CoV-2). To infect the cells, the virus relies on a structural protein (Spike) that recognizes the angiotensin-converting enzyme 2 (ACE2) cell receptor (1, 2), which is expressed in a wide range of tissues, including the thyroid gland (3, 4).

Much has been done in the search for factors that could minimize or aggravate the severity and mortality of COVID-19 infection. An aspect that has been extensively studied is how obesity and metabolic abnormalities affect the outcome of COVID-19 infection, with the resulting consensus that visceral adiposity, low muscle mass, and high concentration of intramuscular fat (myosteatosis) are independent risk factors for critical illness and mortality (5, 6).

While looking for independent metabolic factors that affect the severity of the illness and mortality, a prospective study with 220 consecutive patients with moderate-to-severe COVID-19 revealed that heterozygosity for the Thr92Ala-DIO2 gene was associated with reduced severity of the disease and mortality (7). The DIO2 gene encodes the type 2 deiodinase (D2), the critical enzyme that converts the pro-hormone T_4 to its active form, T_3. At least one Thr92Ala-DIO2 (rs225014) allele can be found in about 50% of the population worldwide; carrying it is associated with an approximately 40% reduction in the conversion of T_4 to T_3 (8, 9, 10).

Several studies have linked the Thr92Ala-DIO2 polymorphism to chronic diseases (such as type 2 diabetes mellitus (11), insulin resistance (12), obesity (13), arterial hypertension (14), osteoporosis (15), and dementias (16)) and a worse prognosis for COVID-19. A recent meta-analysis of 21 studies with more than 20,000 patients confirmed that Thr92Ala-DIO2 heterozygosity is associated with improved long-term outcomes in diabetes, obesity, ischemic stroke, myocardial infarction, and left ventricular hypertrophy (7).

The mechanisms underlying the protective effect of the Thr92Ala-DIO2 heterozygosity remain elusive but could be related to its role in endoplasmic reticulum stress, inflammation, oxidative stress, apoptosis, and mitochondrial dysfunction (9), all pathways linked to the pathophysiology of COVID-19. In addition, the fact that DIO2 is expressed in macrophages (17, 18) could interfere in the immune response to COVID-19 infection (19, 20) and in the outcome in hospitalized COVID-19 patients (21, 22, 23).

The association between sarcopenia and myosteatosis with a worse COVID-19 prognosis is notable (5, 6), given that the skeletal muscle is a key target for thyroid hormones (THs). T_3-signaling in skeletal muscle regulates proliferation, metabolism, differentiation, homeostasis, and growth and also plays a key role in muscle protein breakdown (24). Given that T_3 signaling in the skeletal muscle can be modulated by DIO2, in the present study, we tested whether the better COVID-19 outcomes observed in heterozygous carriers of the Thr92Ala-DIO2 polymorphism is associated with an effect on visceral, subcutaneous fat, area, and muscle density.

Materials and methods

Subjects and data collection

The present study was a subgroup analysis of a clinical trial designed to assess thyroid dysfunction and DIO2 polymorphism in COVID-19 in-hospital patients (7, 21). This was a prospective cohort study that lasted between June and August 2020 and included 172 consecutive patients with confirmed COVID-19 admitted to the emergency department of the Metropolitan Hospital Dom José Maria Pires, a tertiary referral hospital in João Pessoa, Paraíba, Brazil (Fig. 1). The study was approved by the Human Research Ethics Committee of the Lauro Wanderley University Hospital (CAAE:31562720.9.0000.5183). This study was performed in agreement with the Declaration of Helsinki and local and national regulations. Written consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Inclusion and exclusion criteria

Blood samples (50 mL) were collected while patients were in the emergency department within the first 48 h of admission. One hundred seventy-two consecutive patients with a positive nasopharyngeal swab result

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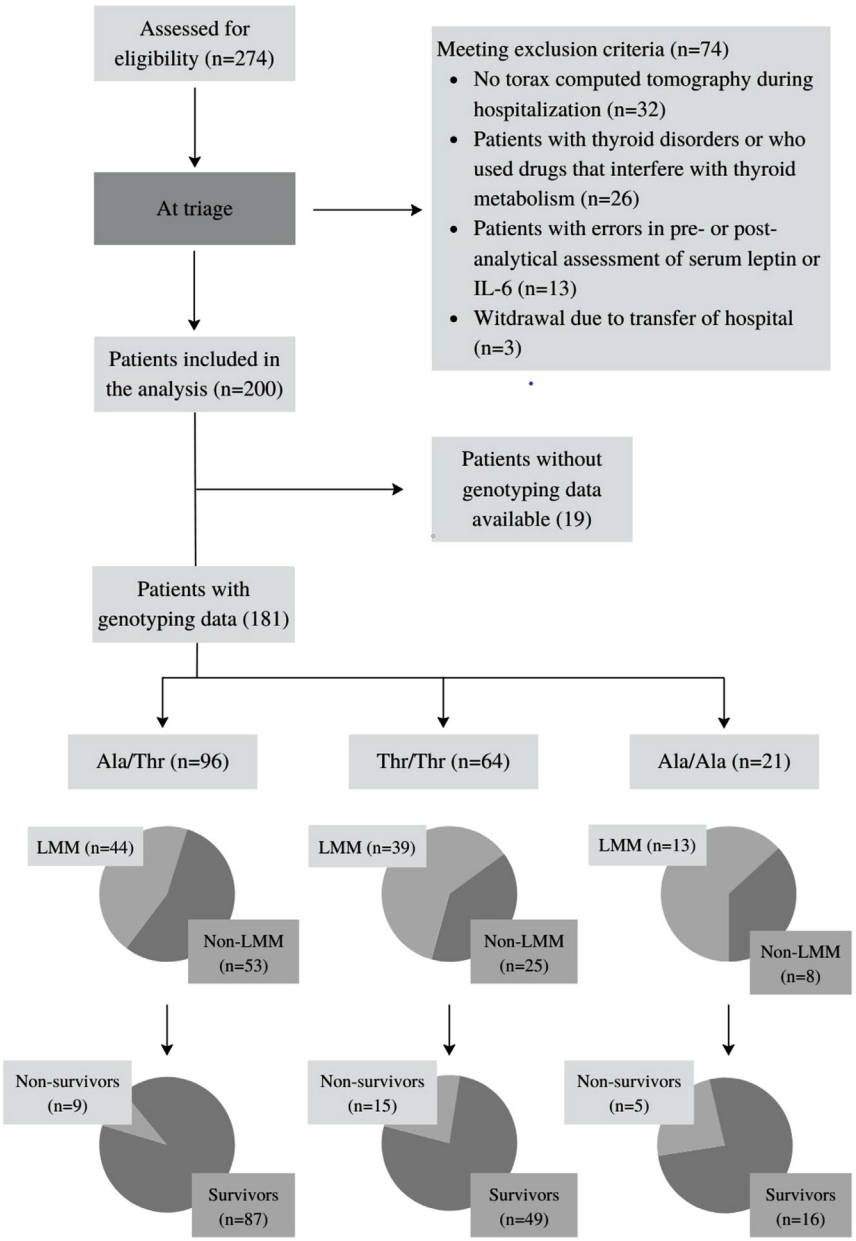

Figure 1

Flowchart of the study. IL-6, interleukin 6; LMM, low muscle mass.

(RT-qPCR – Biomol OneStep/COVID-19, IBMP, Paraná, Brazil) for SARS-CoV-2 were included. We also included patients with negative RT-qPCR if all the following criteria were met: clinical, radiological, and serological (IgG positive for SARS-CoV-2). We exclude patients with a history of thyroid disease, diagnosis of pregnancy, and who used iodinated contrast in the last 6 months or drugs that interfere with TH metabolism.

Outcomes

The primary objective was (1) to identify a potential association between Thr92Ala-DIO2 polymorphism and body composition (appendicular muscle mass, myosteatosis, and fat distribution) and (2) to test whether the improved COVID-19 outcomes observed in heterozygous carriers of the Thr92Ala-DIO2 polymorphism depend on an association with body composition.

Exploratory analyses included cumulative mortality, blood biochemistry, thyroid function tests, comorbidities, complications, and severity scores during admission according to Thr92Ala-DIO2 polymorphism and body composition.

Procedures

The detailed clinical information of each patient was obtained by physicians using a standard questionnaire upon admission, including sociodemographic

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information, medical history, laboratory findings, and previous treatments. Patient severity on admission was first quantified using three severity scoring: the quick Sepsis-related Organ Failure Assessment (qSOFA), the National Early Warning Score 2 (NEW2), and the chest CT severity score (25).

We split the cases into two clinical classifications: severe and critical. Severe cases met any of the following criteria: respiratory rate > 30 cycles/min, oxygen saturation < 93% at rest, partial arterial pressure of oxygen (PaO_2)/concentration of oxygen (FiO_2) < 300 mm Hg (1 mm Hg = 0.133 kPa), and the extent of lung injury (ground-glass opacity) estimated > 50%. Critical cases met any of the following criteria: a manifestation of respiratory failure requiring mechanical ventilation, presence of shock, and other organic failures that need follow-up and treatment in an intensive care unit (ICU). Blood samples for patients who met the inclusion criteria were collected before interventions or therapy that could potentially interfere with or alter TH or cytokine serum levels, always performed within the first 48 h of admission.

Serum biochemistry

Plasma concentrations of interleukin 6 (IL-6), high-sensitive C-reactive protein (CRP), D-dimer and lactate dehydrogenase (LDH), thyroid-stimulating hormone (TSH), free triiodothyronine (fT_3), free thyroxine (fT_4), reverse triiodothyronine (rT_3), thyroglobulin, anti-thyroid peroxidase antibodies (anti-TPO), and ferritin were assessed using chemiluminescence immunoassay (MAGLUMI-2000-PLUS, Shenzhen New Industries Biomedical Engineering Co., Shenzhen, China), according to the manufacturer's protocol. The complete blood cells count with differential was performed on a MEK-7300 hematological analyzer (Nihon Kohden®, Tokyo, Japan). The neutrophil-to-lymphocyte ratio (NLR) was calculated by the absolute neutrophil count divided by the absolute lymphocyte count.

Image analysis

Chest CT scans were performed on a 64-detector CT scanner (Revolution EVO, General Electric). Images were acquired in the supine position after end-inspiration and extended from the lung apices to the costophrenic angles by using the following parameters: 120 kV, 350 mAs, rotation time 0.4 s, pitch 1.5, and slice thickness, 2–5 mm. The technical parameters of CT acquisition were adjusted according to the clinical problem under investigation and the patient body size. CT scans of the thorax were used to diagnose suspected SARS-CoV-2 pneumonia. We considered the following thoracic CT patterns: (1) ground-glass opacities, (2) consolidation, (3) crazy-paving sign, (4) reticulation, and (5) the prominent pattern of opacities (according to the extent of involvement). In all cases, we conducted a semiquantitative CT severity score proposed by Pan *et al.* (25).

CT scans were also used to quantify the subcutaneous (SAF), visceral abdominal fat (VAF), and MA (abdominal muscles excluding the psoas muscle) areas. Although magnetic resonance imaging (MRI) would have been superior, it was not feasible under the local circumstances complicated by the severity of the patient's conditions. Our utilization of CT in these studies was based on Faron *et al.* (2020), who concluded that CT can be used to quantify skeletal muscle fat content similarly to MRI's proton density fat fraction (26), particularly those involving patients with sarcopenia. This analysis was performed by an experienced radiologist (with over 10 years of experience) using a CT scanner by the AW VolumeShare (27, 28), and we performed semi-automated segmentation using 3D-Slicer Software (version 4.11.0, www.slicer.org) (2020) with a method previously described (29). We analyzed the cross-sectional tissue areas using tissue-specific Hounsfield Units (HUs) attenuation ranges. We used the following literature values: (1) VAF and SAF: between –50 and 250 HU and (2) MA: between –29 and 150 HU. The first slice in which the lung bases were no longer visible at the thoracoabdominal level (between the twelfth thoracic vertebra (T12) – second lumbar vertebra (L2)) was selected for the analysis. Data for the selected tissue, including surface area, were expressed in square centimeters (cm^2). Skeletal muscle radiation attenuation (SM-RA) was computed as the mean HU value of all pixels included in MA. The relative distribution of abdominal adipose tissue was assessed using the VAF, SAF, SM-RA, and VAF/MA ratio (Supplementary Figure 1, see section on supplementary materials given at the end of this article).

Genotyping

DNA was extracted from peripheral blood leukocytes by a standardized salting out procedure. Thr92Ala-DIO2 (rs225014) polymorphism was found using primers and probes contained in the Human Custom TaqMan® Genotyping Assay (7500 Real-Time PCR Systems, Applied Biosystems, Foster City, CA). Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 1.3; Applied Biosystems). We successfully genotyped 181 patients for both polymorphisms. All amplification reactions were performed twice. The genotyping success was >95%, with a calculated error rate based on PCR duplicates of 0.01%.

Statistical analysis

We predicted with Gpower 3.1.9.7 software the total number of patients to ensure a power of 0.95 for *F* tests targeting a large effect size ($f = 0.3$). Chi-squared tests were used to determine whether samples were in Hardy–Weinberg equilibrium. Variables with a non-normal distribution are expressed as median (interquartile range). We used the independent *t*-test for comparisons between groups of normally distributed variables and the Mann–Whitney *U* test for comparisons

between groups of non-normally distributed variables. The data were expressed as median ± IQR. We used Kruskal–Wallis test analysis followed by Dunn's *post hoc* test with Benjamini–Hochberg multiple comparison corrections. Mann–Whitney, chi-square, or Cochran–Armitage tests were used for non-parametric variables. We used the Kaplan–Meier method and the log-rank test to investigate the relationship between variables: MA, myosteatosis, and COVID-19 prognosis.

We used uni- and multi-variate logistic regression analysis on the whole group (172 patients) to investigate the potential association between the heterozygous allele (Thr/Ala) vs the homozygous alleles (Thr/Thr and Ala/Ala) with low muscle mass and myoesteatosis. Five multivariate logistic regression models estimated the odds of low muscle mass and myosteatosis. The first model (model 1) included sociodemographic and clinical features: age >60 years, male gender, diabetes, low SAF, high VAF, and obesity. The second and third models (models 2 and 3) aimed to evaluate laboratory tests; model 2 (assessed the thyroid function: TSH, fT_4, fT_3, and rT_3); model 3 (analyzed markers of inflammation, tissue damage, and hemochromocytometric parameters: IL-6, CRP, red cell distribution width (RDW), creatine, neutrophils, and LDH). Finally, model 4 was adjusted for models 1, 3, and 5, with all variables of the analyzed models.

The significance level of $P < 0.05$ was accepted as statistically significant. We used the statistical program GraphPad Prism, v.7.00 (2016) to perform the statistical tests.

Results

A total of 274 adult patients admitted with COVID-19 were eligible to participate in the study. After applying the inclusion and exclusion criteria, 200 patients were enrolled in the study. An additional 19 patients were excluded for lack of genotype determination. The remaining 181 patients completed the study (Fig. 1). The median age was 61 (IQR: 49–73) years, and 111 patients (61.3%) were male. The average length of stay in the hospital was 6.0 days (IQR: 4–10), with 43 (23.8%) patients being admitted to the ICU, and 29 (16%) deaths.

The 181 patients were stratified into three subgroups according to the genotype: Thr/Thr ($n = 64$), Thr/Ala ($n = 96$), and Ala/Ala ($n = 21$) (Fig. 1). The Thr allele frequency was 0.62 and the Ala allele frequency was 0.38, with distribution in Hardy–Weinberg equilibrium ($P = 0.094$; chi-squared test and Fisher's exact test). Ala/Thr patients were compared with patients carrying the Ala/Ala or the Thr/Thr genotypes.

Low muscle mass and death were less prevalent in heterozygous patients (Thr/Ala) than in homozygous patients (Thr/Thr + Ala/Ala) (Table 1). There were no significant differences between the risk factors evaluated (age, arterial hypertension, diabetes mellitus, heart disease, obesity, and chronic obstructive pulmonary disease) among the three subgroups.

Several thyroid function tests and markers of inflammation, tissue damage, or hemochromocytometric parameters were evaluated across alleles and MA. Only serum fT_3 and RDW levels were influenced by the patient's genotype (Table 2).

Clinical outcomes

The prevalence of low muscle mass was 52.5% (95/181). Low muscle mass was less frequent in Ala/Thr patients (44.8%) than in Thr/Thr (60.9%) or Ala/Ala patients (61.9%) ($P = 0.027$) (Table 1). In addition, MA (97.8 cm² vs 86.5 cm², $P = 0.025$) and myosteatosis (40.2 HU vs 36.3 HU, $P = 0.002$) were higher in the Thr/Ala allele subgroup than in the Thr/Thr + Ala/Ala alleles subgroup (Table 2 and Fig. 2). Among serum TH levels, only TSH and free T_3 levels, and free $T_3 \bullet rT_3$ product were significantly different as a function of MA (Supplementary Figure 2).

When comparing patients with different body compositions (MA < 92 cm² or MA > 92 cm²) and genotypes, age, VAF, SAF, VAF/SAF, MA, VAF/MA, SM-RA, D-dimer, TSH, fT_3, and $fT_3 \bullet rT_3$ were significantly different among the groups (Fig. 3). Logistic regression analysis confirmed that the Thr/Ala allele was associated with a reduced risk of low muscle mass and myosteatosis compared with Thr/Thr + Ala/Ala (overdominant model), even after correcting for 14 comorbidities and other covariates (Fig. 3).

The mortality rate was higher in the homozygotic sarcopenic group (MA < 92 cm²) than in the heterozygous without sarcopenia (34.6% vs 3.7%, $P < 0.0001$) (Fig. 4A). Kaplan–Meier curves showed that patients with sarcopenia (MA < 92 cm²) and homozygosity had lower survival rates ($P = 0.0012$) than the other groups. Notably, the heterozygotes with MA ≥ 92 cm² exhibited the best survival rate. Furthermore, no differences in survival were observed between heterozygotes and homozygotes with normal muscle mass (MA ≥ 92 cm²) (Fig. 4B). Mortality rates were higher in the homozygotic group with myosteatosis (<38 HU) compared to the heterozygous group with myosteatosis (<38 HU) (25% vs 3%, $P < 0.037$), as evidenced by both the Kaplan–Meier curve and Chi-square evaluation. However, no differences in mortality rates were observed between the other groups (Fig. 4C and 4D).

Discussion

Sarcopenia, myosteatosis, and obesity are important risk factors for mortality among older adult COVID-19 patients (6, 30). These conditions are multifactorial processes that involve low-grade chronic inflammation, stem cell exhaustion, increased cellular apoptosis, endothelial, hormonal, and mitochondrial dysfunction (31, 32). To our knowledge, this is the first study to identify

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Table 1 Demographic and clinical characteristics of the cohort in patients and their association with Thr92Ala polymorphism. Data are presented as *n* (%) or as median (IQR). Mann–Whitney test was performed for continuous variables (age, NEWS2, qSOFA, and CT COVID-19 score) while Cochran–Armitage test was performed for all other variables.

	Total	Thr/Ala	Thr/Thr	Ala/Ala	*P*	Thr/Thr + Ala/Ala	*P*
n	181	96	64	21		85	
Age (years)	61 (49–73)	58 (47–73)	62 (50–72)	65 (51–77)	0.541	63 (50–73)	0.309
Age > 60 years	93 (51.4)	46 (47.9)	34 (53.1)	13 (61.9)	0.232	47 (55.3)	0.321
Gender male	111 (61.3)	65 (67.7)	33 (51.6)	13 (61.9)	0.183	46 (54.1)	0.061
Length of hospital stay (days)	6 (4–10)	6 (4–9)	7.5 (4–11.7)	6 (4.5–12.5)	0.532	7 (4–12)	0.278
Symptom onset to hospital admission (days)	9 (7–11)	10 (7–11)	9 (6.2–10.7)	7 (5–11)	0.342	9 (6–10.5)	0.212
Comorbidities							
Hypertension	118 (65.2)	57 (59.4)	47 (73.4)	14 (66.7)	0.181	39 (40.6)	0.080
Diabetes mellitus	85 (47)	47 (49)	30 (46.9)	8 (38.1)	0.414	38 (44.7)	0.567
Heart disease	21 (11.6)	11 (11.5)	9 (14.1)	1 (4.8)	0.661	10 (11.7)	0.948
Chronic pneumopathy	9 (5)	4 (4.2)	4 (6.3)	1 (4.8)	0.717	5 (5.9)	0.596
Obesity	89 (49.2)	47 (49)	33 (51.6)	9 (42.9)	0.808	42 (49.4)	0.951
Low muscle mass	95 (52.5)	43 (44.8)	39 (60.9)	13 (61.9)	0.043	53 (55.2)	0.027
Complications							
Use of vasoactive drugs	21 (11.6)	9 (9.4)	9 (14.1)	3 (14.3)	0.362	12 (14.1)	0.994
Death	29 (16)	9 (9.4)	15 (23.4)	5 (23.8)	0.018	20 (23.5)	0.009
Admission to the ICU	43 (23.8)	19 (19.8)	19 (29.7)	5 (23.8)	0.333	24 (28.2)	0.182
Scores systems							
NEWS2 score	6 (5–7)	6 (5–7)	5 (5–6)	6 (5–7)	0.245	6 (5–7)	0.321
q-SOFA score	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	0.731	1 (1–1)	0.538
CT COVID-19 score	20 (15–20)	20 (15–20)	20 (15–20)	20 (15–20)	0.883	20 (15–20)	0.951

CT, computed tomography; ICU, intensive care unit; NEWS2, National Early Warning Score 2; qSOFA, quick Sepsis Related Organ Failure Assessment.

increased muscle mass and reduced myosteatosis in heterozygous COVID-19 patients carriers of the Thr92Ala-DIO2 polymorphism. This was detected through robust univariate and multivariate logistic regression analyses, adjusted for multiple (14) covariates. The importance of these findings is linked to the protective effect of Thr92Ala-DIO2 heterozygosity on COVID-19 mortality (7). Here, we assessed clinical outcomes in COVID-19 patients considering reduced muscle mass, myosteatosis, and Thr92Ala-DIO2 heterozygosity. Remarkably, we observed that the protective effect of Thr92Ala-DIO2 heterozygosity was restricted to the patients who had reduced muscle mass (heterozygosity for Thr92Ala-DIO2 had no effect in patients that had normal muscle mass), and it was only minimally affected by myosteatosis. No association between heterozygosity for Thr92Ala-DIO2 and visceral obesity were observed.

Skeletal muscle is a primary target of TH signaling, influencing structural and metabolic properties; thus, several studies have addressed the role of TH in muscle health (33) There is clear evidence that an excess of TH leads to accelerated proteolysis and reduction in muscle mass. For example, Brennan *et al.* (2006) documented significant improvements in thigh strength and cross-sectional area in patients with overt hyperthyroidism ($n = 30$) and subclinical hyperthyroidism ($n = 24$) 6–9 months after they achieved euthyroidism, highlighting

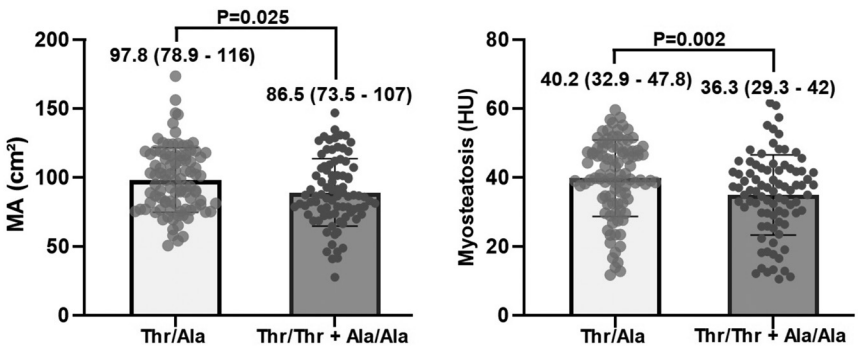

Figure 2

Thr92Ala-DIO2 polymorphism and tomographic parameters (heterozygous, and MA < 92 cm²) in 172 COVID-19 hospitalized patients during the first 48 h of admission. Gray areas in plots represent normal reference ranges. Statistics used: Mann–Whitney test. HU, Hounsfield units; MA, muscle area.

Table 2 Tomographic and laboratory variables evaluated in non-critical and critical patients and their association with Thr92Ala polymorphism. Mann–Whitney test, Kruskal–Wallis test, ANOVA, and Benjamini–Hochberg's (B-H) test were performed for all variables.

	RR	Total	Thr/Ala	Thr/Thr	Ala/Ala	*P* and B-H test	Thr/Ala vs Thr/Thr + Ala/Ala	*P*
n		181	96	64	21		85	
VAF (cm^2)		128 (87–179)	125 (87–179)	128 (93–176)	137 (79–192)	0.953	129 (86–181)	0.801
SAF (cm^2)		150 (96–212)	146 (87–216)	153 (117–213)	157 (61–188)	0.376	157 (104–206)	0.946
MA (cm^2)		90.6 (77–112)	97.8 (78.9–116)	86.4 (73.3–106)	87.2 (75.8–108)	0.04*	86.5 (73.5–107)	0.025
VAF/SAF		0.87 (0.56–1.3)	0.86 (0.56–1.4)	0.82 (0.51–1.30)	1.1 (0.62–1.4)	0.505	0.87 (0.54–1.32)	0.837
VAF/MA		1.47 (0.96–1.97)	1.34 (0.96–1.8)	1.59 (0.96–2.1)	1.57 (1.04–2)	0.283	1.58 (0.97–2.04)	0.113
SM-RA (HU)		38.5 (30.3–46.2)	40.2 (32.9–47.8)	34.9 (29.2–41.7)	37 (28–43.6)	0.007†	36.3 (29.3–42)	0.002
TSH (μIU/mL)	0.4–5.8	1.62 (0.90–3.00)	1.66 (0.81–2.99)	1.34 (0.88–2.87)	2.15 (1.00–3.98)	0.214	1.55 (0.9–3.0)	0.598
fT_4 (ng/dL)	0.89–1.72	1.30 (1–1.67)	1.29 (1.01–1.69)	1.30 (1.03–1.57)	1.28 (0.87–1.62)	0.773	1.30 (0.99–1.69)	0.753
fT_3 (pg/mL)	2.0–4.2	2.99 (2.6 – 3.4)	3.12 (2.66–3.48)	2.95 (2.64–3.50)	2.84 (2.16–3.28)	0.229	2.92 (2.6 –3.38)	0.173
rT_3 (ng/mL)	0.1–0.35	0.49 (0.30–0.66)	0.49 (0.29–0.66)	0.45 (0.31–0.66)	0.53 (0.40–0.65)	0.652	0.49 (0.31–0.66)	0.928
Leptin		4.4 (1.5–8.4)	4.2 (1.3–7.7)	5.1 (1.7–10.4)	3.4 (1.6–5.1)	0.460	4.5 (1.7–9.4)	0.920
IL-6 (pg/mL)	<3.4	49.8 (23.1–95.9)	50.3 (23–108)	48 (25.2–91.7)	34 (19.1–91.3)	0.798	43.1 (22.9 – 89.3)	0.624
D-dimer (ng/mL)	<500	732 (478–1679)	714 (440–1525)	878 (478–1592)	765 (511–3723)	0.363	842 (490–1775)	0.285
LDH (U/L)	207 - 414	761 (548–1013)	777 (551–1032)	760 (547–1009)	718 (465–1002)	0.829	742 (530–1006)	0.732
Creatinine (mg/dL)		1.10 (0.9–1.38)	1.1 (0.88–1.35)	1.1 (0.9–1.42)	1.0 (0.9–1.32)	0.448	1.1 (0.90–1.39)	0.633
CRP (mg/dL)	<5.0	84 (37.6–154)	93.8 (41.2–154)	77.2 (28.4–156)	76.8 (36.8–150)	0.690	77.2 (30.1–155)	0.391
Neutrophil (10^3 cells/μL)	1935–6700	7303 (5384–10069)	7116 (5460–10754)	7701 (5204– 9474)	7820 (5859–10165)	0.905	7820 (5313–9614)	0.782
RDW (%)	11–14	13.8 (13.4–14.2)	13.8 (13–14)	13.7 (13–14)	13.9 (13.3–14.2)	0.565	13.8 (13.4–14.2)	0.433
N/L ratio	1–3	9.3 (6–14.6)	8.8 (5.6–14)	10.2 (6.2–15)	10.8 (6.6–14.5)	0.509	10.4 (6.6–15)	0.248
Albumin (g/dL)	3.5–5.5	3.3 (2.9–3.7)	3.3 (2.9–3.7)	3.4 (2.9–3.7)	3.2 (2.7–3.4)	0.409	3.3 (2.9–3.7)	0.684

*A-B ($P = 0.025$); †A-B ($P = 0.006$).

CRP, C-reactive protein; fT3, free triiodothyronine; fT4, free thyroxine; IL-6, interleukin 6; IQR, interquartile range; LDH, lactate dehydrogenase; MA, muscle area; N/L ratio, neutrophil-lymphocyte ratio; OR, odds ratio; RDW, red cell distribution width; RR, reference range; SAT, subcutaneous abdominal fat area; VAF, visceral abdominal fat area; SM-RA, skeletal muscle radiation attenuation.

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	Ala/Thr *vs.* Ala/Ala+Thr/Thr (Overdominat model)					
	Low muscle mass (MA < 92 cm²)			Myosteatosis (SM-RA < 38 HU)		
	OR*	CI 95%	P	OR*	CI 95%	P
Model 1 – Age > 60 year	0.53	0.28 – 0.98	0.047	0.28	0.13 – 0.57	0.0005
Model 1 – Gender (male)	0.59	0.30 – 1.16	0.128	0.35	0.19 – 0.66	0.0014
Model 1 – Diabetes	0.50	0.27 – 0.91	0.023	0.31	0.16 – 0.58	0.0002
Model 1 – Low SAF	0.50	0.27 - 0.91	0.026	0.33	0.18 - 0.60	0.0004
Model 1 – High VAF	0.49	0.26 – 0.90	0.024	0.32	0.17 – 0.60	0.0003
Model 1 – Obesity	0.46	0.24 – 0.87	0.018	0.32	0.17 – 0.59	0.0003
Model 1	0.57	0.26 – 1.22	0.153	0.29	0.13 - 0.60	0.0011
Model 2 – TSH	0.50	0.27 – 0.92	0.028	0.32	0.17 – 0.59	0.0004
Model 2 – Free T_3	0.53	0.29 – 0.97	0.041	0.33	0.18 – 0.61	0.0005
Model 2 – Free T_4	0.51	0.28 – 0.93	0.029	0.33	0.17 – 0.60	0.0004
Model 2 – Reverse T_3	0.52	0.28 – 0.95	0.035	0.33	0.18 – 0.61	0.0004
Model 2	0.52	0.27 – 0.97	0.041	0.33	0.17 – 0.61	0.0005
Model 3 – Leptin	0.51	0.28 – 0.92	0.028	0.33	0.17 – 0.60	0.0003
Model 3 – IL6	0.52	0.28 – 0.94	0.033	0.32	0.17 – 0.60	0.0004
Model 3 – CRP	0.50	0.27 – 0.93	0.031	0.37	0.19 – 0.70	0.002
Model 3 – RDW	0.48	0.26 – 0.88	0.019	0.34	0.18 – 0.64	0.0008
Model 3 – Creatin	0.49	0.26 – 0.90	0.022	0.35	0.18 – 0.64	0.0009
Model 3 – Neutrophil	0.51	0.28 – 0.92	0.027	0.32	0.17 – 0.60	0.0004
Model 3 – LDH	0.46	0.25 – 0.85	0.014	0.29	0.15 – 0.54	0.0001
Model 3	0.41	0.20 - 0.80	0.01	0.38	0.19 – 0.76	0.006
Model 4	0.39	0.15 - 0.97	0.046	0.31	0.13 – 0.69	0.005
Model 5	0.31	0.11 - 0.81	0.021	0.30	0.13 – 0.69	0.0051

Figure 3

Multivariable regression analyses between D2 Thr92Ala polymorphism (Thr/Thr, Thr/Ala, Ala/Ala, and overdominant model) and low muscle mass and myosteatosis. Multivariable regression analyses – model 1: adjusted for age > 60 anos, diabetes, low SAF, high VAF, and obesity; model 2: adjusted for TSH, fT3, fT4, and rT3; model 3: adjusted for leptin, IL6, CRP, RDW, neutrophil, and LDH; model 4 – adjusted for models 1 and 3; model 5 – adjusted for all of the abovementioned variables.

the critical importance of early thyroid management, particularly in vulnerable populations such as the elderly (34). This indicates that an excess of TH can have significant consequences to muscle mass. Nonetheless, a study by Netzer *et al.* (35) on 267 older adults with persistent subclinical hypothyroidism revealed that LT4 treatment did not significantly affect gait speed, handgrip strength, or annual muscle mass change when compared to a placebo (35).

Several lines of evidence indicate that DIO2 plays a role in skeletal muscle differentiation and growth (36), which could explain its relationship with skeletal muscle mass. DIO2 expression is typically low in muscle fibers but increases in muscle stem cells during myogenesis and regeneration, supplying additional T3 that promotes differentiation (36, 37). DIO2 may also play a role in skeletal muscle regeneration as shown in mice during the recovery process (post-lesion) when DIO2 is expressed in fibro-adipogenic progenitor cells (38). Moreover, DIO2 is induced in skeletal muscle during physical exercise and is associated with the induction of PGC-1a expression, linking Dio2 to energy homeostasis in muscle tissues (39).

Recent studies highlight the complex interactions between DIO2 polymorphisms and wider genetic networks. McAninch *et al.* (2015) revealed correlations between different DIO2 alleles and the expression of 81 genes associated with inflammatory processes, oxidative stress, and neurodegenerative diseases. Notably, the Thr/Ala genotype showed associations with genes such as CXCR4, SLC16a2, SLC44a2, CDK2, and BST2 (40). Research

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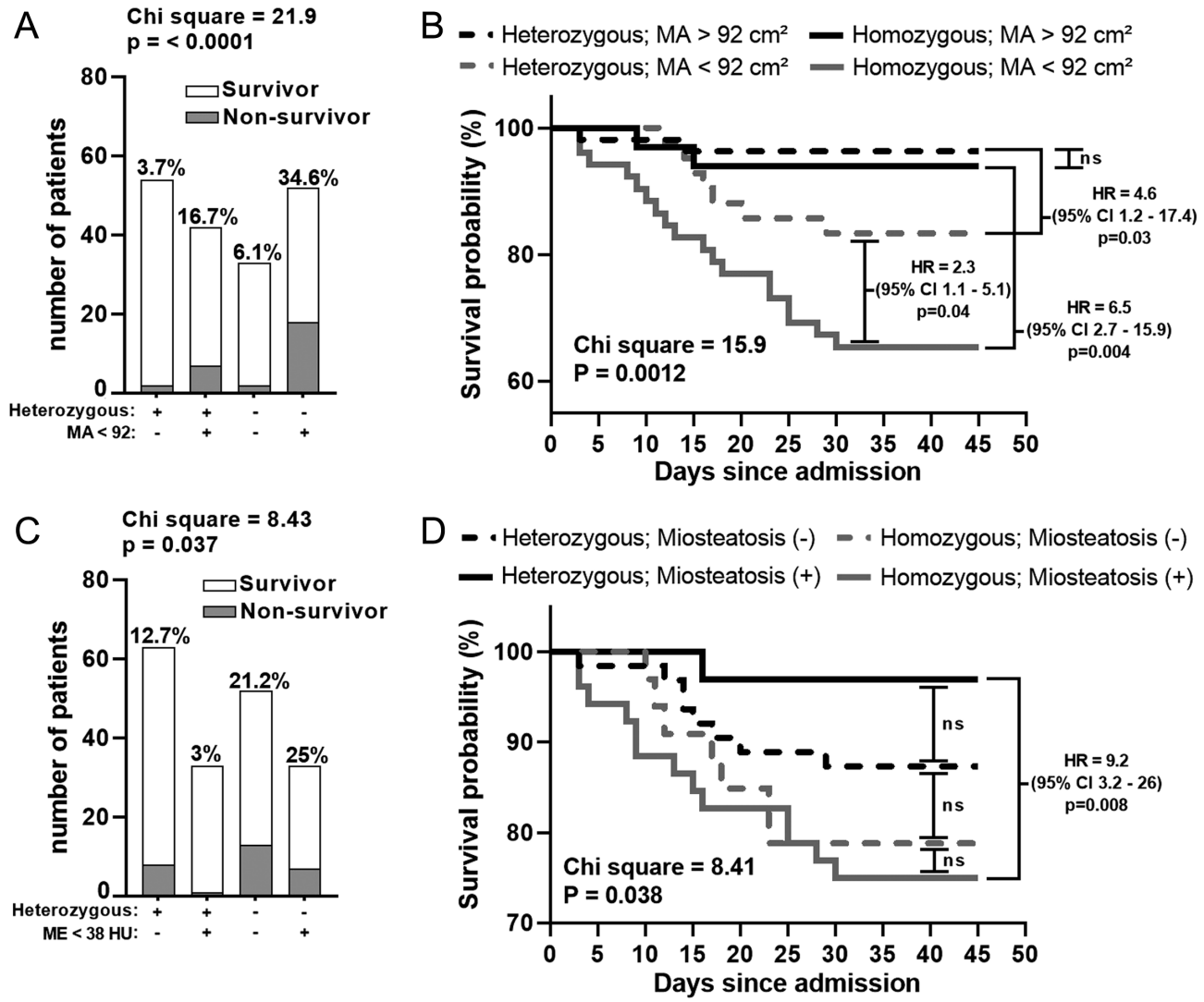

Figure 4

Kaplan–Meier curves and Bar chart for predicting mortality in patients with COVID-19 (heterozygous, and MA < 92 cm², and ME < 38 HU). (A) and (C) Bar chart depicting sample number with (+) and without (−) the parameter below the cutoff (heterozygous, MA < 92 cm², and ME < 38 HU) in patients with COVID-19 (survivors vs nonsurvivors) and highlighting the proportion of nonsurvivor. (B) and (D) Kaplan–Meier curves for predicting mortality in patients with COVID-19 (heterozygous, MA <92 cm², and ME < 38 HU). HR, hazard ratio; HU, Hounsfield units; MA, muscle area; ME, myosteatosis; ns, not significant.

in Slc44a2 knock-out mice showed that a decrease in muscle mass and tone appeared to increase muscular thyroid hormone content (41, 42). Additionally, Shams *et al.* (43) demonstrated the indispensable role of CXCR4 signaling in the early activation, proliferation, and self-renewal of satellite cells for skeletal muscle recovery during acute events (43). These findings suggest an indirect link between the Thr92Ala-DIO2 heterozygosity and muscle mass maintenance, providing a potential mechanistic pathway through which this polymorphism may confer a protective effect in COVID-19.

The present study is not without some limitations. They include (i) a relatively small number of patients, which has an effect size index of 0.3; (ii) an analysis that was limited to hospitalized moderate-to-severe COVID-19 patients, which may not apply to individuals with non-hospitalized COVID-19 patients; (iii) analysis of the skeletal muscle that was limited to area and the presence of fat. In addition, it is conceivable that the COVID-19 infection could have modified the skeletal muscle mass, the presence of myosteatosis and/or presence of visceral obesity. Nonetheless, the median interval between start of symptoms and admission was 9 days (IQR: 7–11) (Table 1) and the CT scans were done within 48 h of hospital admission, minimizing the chances that poor COVID-19 outcomes affected the skeletal muscle.

In conclusion, here we found that the Thr92Ala-DIO2 heterozygosity is associated with increased skeletal

muscle mass and less myosteatosis in COVID-19 patients. In addition, the protective effect of carrying a Thr92Ala-DIO2 heterozygosity on COVID-19 mortality is restricted to patients with reduced muscle mass. Future studies should confirm these findings and clarify their mechanistic basis.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/ETJ-24-0068.

Declaration of interest

AB is a consultant for Abbvie, Acella, Alligos, and Synthonics. The other authors declare that there is no conflict of interest that could prejudice the impartiality of the study reported.

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Author contribution statement

The attributions the authors had in the production of the manuscript were literature review and article writing (FELB, AB, GCV, HER), text review and interpretation of data for the work (DCAB, MCRG, AB, HER), figure creation (FH), data collection (FELB, GC, FLLB, JBO, HSS, HMPT, JLR), and text review (AB, HER, CAVF, RSC) and research coordinator (HER).

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