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Clinical Research Article

Heterozygote Advantage of the Type II Deiodinase Thr92Ala Polymorphism on Intrahospital Mortality of COVID-19

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Abstract

Context: The type 2 deiodinase and its Thr92Ala-DIO2 polymorphism have been linked to clinical outcomes in acute lung injury and pulmonary fibrosis.

Objective: Our objectives were to evaluate were cumulative mortality during admission according to Thr92Ala-DIO2 polymorphism.

Methods: Here we conducted an observational, longitudinal, and prospective cohort study to investigate a possible association between the Thr92Ala-DIO2 polymorphism and intrahospital mortality from COVID-19 in adult patients admitted between June and August 2020. Blood biochemistry, thyroid function tests, length of stay, comorbidities, complications, and severity scores were also studied according to Thr92Ala-DIO2 polymorphism.

Results: In total, 220 consecutive patients (median age 62; 48-74 years) were stratified into 3 subgroups: Thr/Thr (n = 79), Thr/Ala (n = 119), and Ala/Ala (n = 23). While the overall mortality was 17.3%, the lethality was lower in Ala/Thr patients (12.6%) than in Thr/Thr patients (21.7%) or Ala/Ala patients (23%). The heterozygous genotype (Thr/Ala) was associated with a 47% reduced risk of intrahospital mortality whereas univariate and multivariate logistic regression adjusted for multiple covariates revealed a reduction that ranged from 51% to 66%. The association of the Thr/Ala genotype with better clinical outcomes was confirmed in a metanalysis of 5 studies, including the present one.

Conclusion: Here we provide evidence for a protective role played by Thr92Ala-DIO2 heterozygosity in patients with COVID-19. This protective effect follows an inheritance model known as overdominance, in which the phenotype of the heterozygote lies outside the phenotypical range of both homozygous.

Key Words: thyroid, type II deiodinase, polymorphism and COVID-19

Abbreviations: AIDS, acquired immune deficiency syndrome; AIS, acute ischemic strokes; ALI, acute lung injury; ALT, Alanine transaminase; AMI, acute myocardial infarction; anti-TPO, antithyroid peroxidase antibodies; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; BMI, body mass index; BST2, bone marrow stromal antigen 2; CCL, C-C motif chemokine ligand; CDK2, cyclin-dependent kinase 2; CI, confidence interval; COVID-19, coronavirus disease 19; CRP, C-reactive protein; CT, computed tomography; CXCR4, C-X-C chemokine receptor type 4; DIO2, type II deiodinase; DIO3, type III deiodinase; fT3, free triiodothyronine; fT4, free thyroxine; HLA, human leukocyte antigen; HIV, human immunodeficiency virus; HR, hazard ratio; ICU, intensive care unit; IL-6, interleukin 6; IR, interquartile range; IS, ischemic stroke; LDH, lactate dehydrogenase; LVH, left ventricular hypertrophy; M-H, Mantel–Haenszel; NEWS2, National Early Warning Score 2; NTIS, nonthyroidal illness syndrome; OR, odds ratio; qSOFA, Quick Sepsis-related Organ Failure Assessment; RT-qPCR, real-time reverse transcription polymerase chain reaction; SARS, severe acute respiratory syndrome; rT3, reverse triiodothyronine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNP, single nucleotide polymorphisms; SLC44a2, solute carrier family 44 member 2; TH, thyroid hormone; TSH, thyrotropin.

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The coronavirus 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) virus, has created a major global health crisis. Despite having infected hundreds of millions and killed more than 5 million, gaps remain in our understanding of the pathophysiology of the disease (1). SARS-Cov-2 infection can cause pulmonary and systemic inflammation, leading to multiple organ dysfunction in high-risk populations such as elderly people, pregnant women, patients with obesity, hypertension, and diabetes, and those on long-term immunosuppressive therapy (2-5). The lethality among hospitalized patients ranges from 11% and 15% (2, 6, 7).

The most feared complication of COVID-19 is severe acute respiratory syndrome (SARS) which has an in-hospital prevalence of 17% to 29% (2, 7). Acute lung injury (ALI) is 1 of the main causes of respiratory failure that usually develops in response to major insults, such as sepsis, trauma, viral or bacterial pneumonia, and multiple blood transfusions during hospitalization (8). In this regard, it is well accepted that low triiodothyronine syndrome is a strong predictor of poor prognosis in critically ill hospitalized patients, including patients admitted to the intensive care unit (ICU), pneumonia, severe burns, stroke, septic shock, respiratory failure, cardiovascular diseases, and multiple trauma (9-16). This has also been found in patients with COVID-19, with strong association between reduced levels of free T3 (fT3) at admission of hospitalized patients with intrahospital severity and mortality (17-21).

While different strategies have been employed to minimize ALI and improve lung function, we note a growing interest in treating lung disease with thyroid hormone (TH) in pulmonary edema and acute respiratory distress syndrome (ARDS). Instillation of the TH T3 increased alveolar fluid clearance in rat lungs with hypoxia-induced lung injury (22). In addition, TH exhibits antifibrotic properties that are associated with the protection of alveolar epithelial cells and restoration of mitochondrial function. TH can reach the lungs via systemic circulation but can also be activated locally, within type II pulmonary alveolar epithelial cells, through the activity of the iodothyronine deiodinase 2 (DIO2). Remarkably, DIO2 expression and activity were elevated in lungs from patients with idiopathic pulmonary fibrosis, and its knockout worsened bleomycin-induced lung fibrosis in mice (23).

Accordingly, Ma et al reported increased severity of ALI in mice with reduced DIO2 expression (through RNA silencing), suggesting a protective role of DIO2 in ALI. They also reported that carrying a polymorphism in DIO2 (Thr92Ala) in humans confers protection against ALI. Increased DIO2 expression may dampen the ALI inflammatory response, thereby strengthening the premise that TH metabolism is intimately linked to the integrated response to inflammatory injury in critically ill patients (24).

DIO2 is located on the long arm of chromosome 14, at position 14q0.24.3. To date, 6 single nucleotide polymorphisms (SNPs) of these genes have been described in the literature, including rs225014, ORFa-Gly3Asp, rs225010, rs225012, rs2267872, and rs1388378 (25-27). The rs225014 SNP, also known as the Thr92Ala-DIO2 polymorphism, is located in exon 2 of DIO2. It causes the substitution of threonine for an alanine at position 92, which has reduced activity (28, 29). Nonetheless, carrying the Thr92Ala-DIO2 polymorphism does not affect thyroid function tests in individuals who have a normal thyroid gland, but this is controversial in thyroidectomized patients kept on levothyroxine (LT4) (30). In a large report that included approximately 550 LT4-treated patients, the Thr92Ala-DIO2 polymorphism had no effect on the serum T4:T3 ratio (31), but in another series of 140 patients it was associated with a higher T4:T3 ratio (32).

The minor allele Ala92-Dio2 has a high prevalence in the population (38.8-47.6%). Several studies have analyzed its association with multiple chronic diseases, such as type 2 diabetes mellitus (28, 33-35), obesity (36), arterial hypertension (37, 38), osteoarthritis (39), osteoporosis (40), and dementias (41), many of which are risk factors for a worse prognosis for COVID-19 (42-44), but controversy has prevented the formulation of a unifying hypothesis as to its role (29). Here we tested whether the Thr92Ala-DIO2 polymorphism is associated with intrahospital mortality from COVID-19.

Material and Methods

Subjects

This study was performed at the Dom José Maria Pires Metropolitan Hospital (João Pessoa, Paraíba, Brazil). Adults (≥18 years) who presented to the emergency department between June and August 2020 with COVID-19 symptoms and were assessed through blood biochemistry were preliminarily recruited into this observational, longitudinal, and prospective cohort study. 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 in compliance with local and national regulations.

Inclusion and Exclusion Criteria

A total of 220 consecutive patients with a positive result on standard of care reverse transcription polymerase chain reaction test (RT-qPCR; Biomol OneStep/COVID-19, IBMP, Paraná, Brazil) for SARS-CoV-2 in nasopharyngeal swabs, or, in cases of negative RT-qPCR, having clinical, radiological, and serological (IgG positive for SARS-CoV-2) criteria. Samples were centrifuged at 2000*g* for 15 minutes at 4°C and subsequently frozen at −80°C until measurement. Exclusion criteria were (1) patients younger than 18 years, (2) patients with a history of thyroid disease and diagnosis of pregnancy, and (3) patients who had used iodinated contrast in the last 6 months or drugs that interfere with thyroid metabolism. Race or ethnic background were not considered in the study. Although historically the Brazilian population was formed by natives of that country, Europeans, and Africans, the degree to which these races are now intermixed minimizes the relevance of such distinctions in Brazilian populational studies.

Severity Scoring

Within the first 48 hours of admission, patient severity was first defined using 3 scoring systems: (1) the quick Sepsis-related Organ Failure Assessment (qSOFA), the National Early Warning Score 2 (NEW2), and chest computed tomography severity score proposed by Pan et al (45).

Blood Biochemistry

Blood samples (50 mL) were collected within first 48 hours of hospital admission (before interventions or therapy that could potentially interfere or alter TH or cytokine serum

levels, including steroids and heparin). Plasma concentrations of interleukin 6 (IL-6), high-sensitive C-reactive protein (CRP), D-dimer, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH), thyroid function (fT3, free thyroxine [fT4], reverse triiodothyronine [rT3], thyrotropin [TSH]), thyroglobulin, antithyroid peroxidase antibodies (anti-TPO), and albumin were assessed using measured by 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 was calculated by the absolute neutrophil count divided by the absolute lymphocyte count.

Genotyping Analysis

Genomic DNA was extracted from whole blood using standard techniques. In this study the polymorphism was determined by the TaqMan® SNP Genotyping method (7500 Real Time PCR Systems, Applied Biosystems, CA), using the assay for genotyping with TaqMan® probes and primers; in a combination of hybridization and DNA polymerase activity, associated with fluorescence detection (46). For these analyses, a 5-µL solution was be used, containing 20 ng of sample DNA, 2.5 µL of universal Taqman PCR Master Mix (with final concentration 1×), 0.25 µL of the assay (probe and primers) for a concentration of 20×, and 0.125 µL for a concentration of 40× (maintaining a final concentration of 1×), in addition to 2.25 µL of Milli-q® water. For all reactions, a negative and a positive control were performed. For the polymerase chain reaction (PCR), cycles of 10 minutes at 95°C were used for the initial denaturation phase, followed by 50 cycles at 92°C for 15 seconds and 60°C for 90 seconds. We used the software Sequence Detection, version 1.3 (Applied Biosystems, CA) to analyze the data.

Outcomes

Primary

Primary outcomes were cumulative mortality during admission according to Thr92Ala-DIO2 polymorphism.

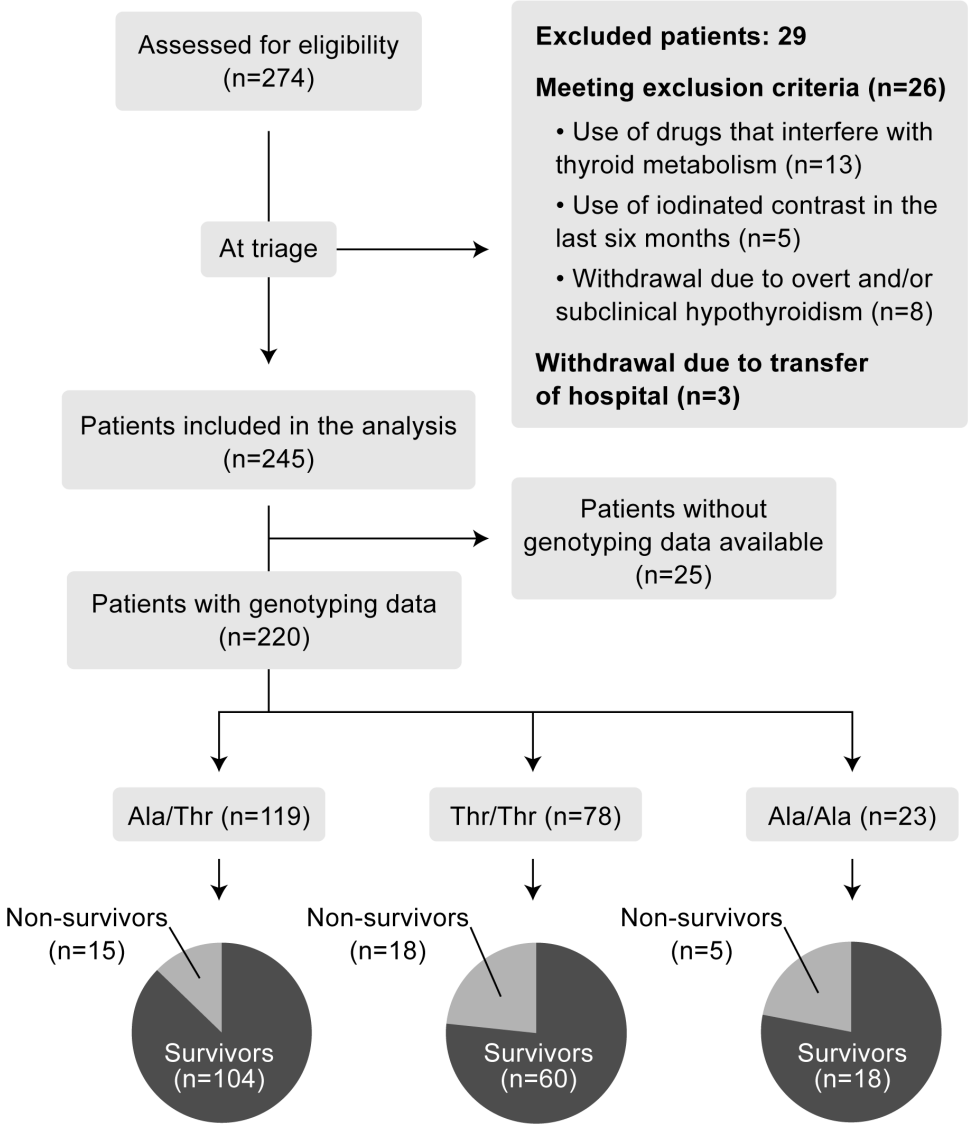

Figure 1. Flowchart of the study.

Secondary

Secondary outcomes were blood biochemistry, thyroid function tests, length of stay, comorbidities, complications, and severity scores according to Thr92Ala-DIO2 polymorphism.

Statistical Analysis

To study mortality, we performed power calculations based on Cohen's method using GPower 3.1.9.7 software. The current sample size showed more than 90% power to detect significance ($\alpha < .05$) in association with allele and haplotype under study conditions, and an effect size index of 0.3 was used. Chi-squared tests were used to determine whether samples were in Hardy–Weinberg equilibrium.

The data were expressed as median ± interquartile range (IQR). We used Kruskal–Wallis test analysis followed by Dunn's post hoc test with Benjamini–Hochberg multiple comparison corrections. Mann–Whitney, chi-squared, or Cochran–Armitage test were used for nonparametric variables. The Kaplan–Meier method and log-rank test were used in our study to investigate the relationship between variables and COVID-19 prognosis.

To assess the relative risk of mortality (odds ratio [OR]), we used univariate and multivariate logistic regression. The significance level of $P < .05$ was accepted as statistically significant. The statistical program GraphPad Prism, v0.7.00 (2016), was used to perform statistical tests.

Next, we used uni- and multivariate logistic regression analysis on the total group (220 patients) to investigate the potential association between the heterozygous allele (Thr/Ala) vs the homozygous alleles (Thr/Thr and Ala/Ala) with mortality. Five multivariate logistic regression models were estimated to individualize mortality by prognostic factors. In the first model (Model 1), the following sociodemographic variables and clinical characteristics were included: male gender, age >60 years, diabetes, arterial hypertension, and length of hospital stay. The second and third models (Model 2 and 3) aimed to evaluate laboratory tests; Model 2 (where the thyroid function was assessed—TSH, fT4, fT3, and rT3) and Model 3 (where markers of inflammation, tissue damage or hemochromocytometric parameters were analyzed—IL-6, CRP, D-dimer, neutrophils, and DHL). Finally, Model 4 was adjusted for Models 1 and 3, and Model 5, for all variables of the analyzed models.

Meta-analysis

A systematic review was conducted according to the recommendations outlined in the PRISMA (47). The electronic databases Pubmed, Cochrane, and SciELO were combed for studies of genetic association between the Thr92Ala-DIO2 polymorphism and diseases. We limited the search to humans

Table 1. Demographic and clinical characteristics of the patient cohort and their association with Thr92Ala-DIO2 polymorphism and mortality (n = 220)

Variables	Mann–Whitney test and Cochran–Armitage test					Univariate logistic regression Mortality		
	Total (n = 220)	Thr/Thr (n = 78)	Thr/Ala (n = 119)	Ala/Ala (n = 23)	P	OR	IC 95%	P
Age (y), median (IQR)	62 (48-74)	63 (51-75)	60 (47-74)	63 (47-77)	.539	1.016	0.993-1.040	.176
BMI(kg/m^2)	29.8 (26-34)	29.8 (27-33)	29.8 (25-34)	29.4 (26-34)	.923	0.993	0.938-1.050	.859
Age > 60 year, n (%)	116 (52.7)	43 (56.5)	60 (50.4)	13 (56.5)	.501	1.467	0.725-3.044	.291
Gender male, n (%)	135 (61.3)	41 (52.5)	79 (66.4)	15 (65.2)	.16	0.647	0.319-1.318	.226
Length of hospital stay (d), median (IQR)	6 (4-10)	7 (4-11)	6 (4-10)	6 (4-12)	.611	1.099	1.052-1.154	<.0001
Comorbidities								
Hypertension, n (%)	144 (65.4)	58 (74.3)	72 (60.5)	14 (60.9)	.049	1.175	0.565-2.559	.672
Diabetes mellitus, n (%)	93 (42.3)	33 (42.3)	51 (42.8)	9 (39.1)	.881	1.352	0.661 -2.755	.405
Cardiopathy, n (%)	28 (12.7)	12 (15.4)	15 (12.6)	1 (4.3)	.628	0.333	0.052-1.187	.146
Chronic pneumopathy, n(%)	10 (4.5)	5 (6.4)	4 (3.3)	1 (4.3)	.317	0.51	0.027-2.891	.540
Neoplasia, n (%)	2 (0.9)	0 (0)	1 (0.8)	1 (4.3)	.631	4.89	0.190-125.5	.265
Obesity, n (%)[a]	103 (49)	37 (49)	55 (49.1)	11 (47.8)	.982	0.39	0.169-0.852	.021
Complications								
NTIS, n (%)	14 (6.4)	4 (5.1)	7 (5.9)	3 (13)	.055	4.1	1.268-12.5	.0142
Cardiovascular shock, n (%)	28 (12.7)	12 (15.4)	13 (10.9)	3 (13)	.357	54.2	19.3-181.4	<.0001
Endotracheal intubation, n (%)	29 (13.2)	14 (17.9)	12 (10)	3 (13)	.111	129.3	39-600	<.0001
ICU admission, n (%)	55 (25)	24 (30.7)	26 (21.8)	5 (21.7)	.37	36.8	14.8-106.9	<.0001
Scores systems								
NEWS2 score, median (IQR)	6 (5 -7)	5 (4-6)	6 (5 -7)	6 (5 -7)	.346	1.162	0.960-1.348	.134
q-SOFA score, median (IQR)	1 (1-1)	1 (1-1)	1 (0-1)	1 (1-1)	.435	1.897	0.892-4.256	.105
CT COVID score, median (IQR)	20 (15-20)	20 (15-20)	20 (15-20)	20 (15-20)	.786	1.043	0.972-1.133	.277

Mann–Whitney test was performed for continuous variables (age, NEWS2, qSOFA and CT COVID score) while Cochran–Armitage test was performed for all other variables.
Abbreviations: BMI, body mass index; CT, computed tomography; ICU, intensive care unit; IQR, interquartile range; NEWS2, National Early Warning Score 2; NTIS, nonthyroid illness syndrome; qSOFA, quick Sepsis-related Organ Failure Assessment.
[a](n = 210 patients).

Table 2. Blood biochemistry in COVID-19 patients and their association with Thr92Ala-DIO2 polymorphism and mortality

Parameters (normal range)	Kruskal–Wallis test median (IQR)						Univariate logistic regression mortality		
	Total (n = 221)	A (Thr/Thr) (n = 79)	B (Thr/Ala) (n = 119)	C (Ala/Ala) (n = 23)	P	Benjamini–Hochberg's test	OR	IC 95%	P
TSH (0.4-5.8 µIU/mL)	1.66 (0.9-3)	1.55 (0.9-3)	1.62 (0.9-2.9)	2.15 (1.0-3.8)	.322		0.986	0.796-1.193	.895
fT4 (0.89-1.72 ng/dL)	1.33 (1-1.6)	1.3 (1-1.5)	1.35 (1.0-1.7)	1.28 (0.8-1.5)	.465		1.011	0.497-1.989	.975
fT3 (2.0-4.2 pg/mL)	2.96 (2.6-3.4)	2.92 (2.6-3.3)	3.14 (2.6-3.5)	2.77 (2.1-3.2)	.029	A-B (P = .054) B-C (P = .023)	0.460	0.276-0.739	.002
rT3 (0.1-0.35 ng/mL)	0.48 (0.3-0.6)	0.38 (0.2-0.6)	0.52 (0.3-0.6)	0.53 (0.3-0.6)	.249		0.086	0.013-0.461	.006
Thyroglobulin (1.59- 59.9 ng/mL)	15.1 (6-26)	15.6 (6-28.7)	14.5 (6-23)	15.8 (6-45)	.725		0.994	0.976-1.005	.414
FT3/RT3	6.60 (4.6-9.9)	7 (4.7-10.6)	6.61 (4.7-9.8)	4.83 (3.7-6.7)	.056	B-C(P = .045) A-C (P = .016)	1.017	0.963-1.067	.496
FT3●RT3	1.28 (0.8-2.1)	1.1 (0.7-1.9)	1.46 (0.8-2.3)	1.24 (0.7-1.9)	.075	A-B (P = .025)	0.441	0.249-0.717	.002
FT4/FT3	0.43 (0.3-0.5)	0.45 (0.3-0.5)	0.43 (0.3-0.5)	0.48 (0.3-0.6)	.691		9.697	1.737-57.4	.01
IL-6 (<3.4 pg/mL)	49.8 (21-87)	43 (23-83)	55.5 (23-96)	32.8 (19-84)	.533		1.000	0.999-1.001	.739
D-dimer (<500 ng/mL)	759 (487-1628)	924 (546-1579)	696 (458-1496)	706 (488-3629)	.447		1.000	1.000-1.000	.047
LDH (207-414 U/L)	742 (538-1014)	723 (522-1006)	772(564-1051)	718 (488-992)	.529		1.001	1.000-1.002	.008
Creatinine (mg/dL)	1.1 (0.9-1.3)	1.13 (0.9-1.4)	1.1 (0.8-1.3)	1.0 (0.9-1.3)	.342		1.017	0.754-1.197	.851
CRP (<5.0 mg/dL)	83 (36-151)	68 (30-145)	100 (42-162)	75.3 (33-142)	.337		1.009	1.004-1.015	.002
ALT (8-42 U/L)	62 (39-102)	53 (38-97)	68 (46-111)	48 (23-114)	.06	A-B(P = .046)	0.992	0.984-0.999	.067
AST (8-42 U/L)	54 (38-80)	49 (36-77)	56 (40-84)	52 (33-75)	.206		0.996	0.988-1.003	.382
Neutrophils (1935-6700 10^3 cells/µL)	7412 (5348-10130)	7701(5237-9290)	7303 (5340-10660)	7560 (5642-9945)	.978		1.000	1.000-1.000	.053
Hemoglobin (13-18 g/dL)	13.4 (12-14.4)	13 (11-14)	13.6 (12-14)	13.3 (12-14)	.032	A-B(P = .009)	0.816	0.680-0.978	.027
N/L ratio (1-3)	9.27 (5.8-14)	9.5 (5.8-14)	9.11 (6-14)	10.5 (5.2-14)	.954		1.077	1.025-1.135	.004
Albumin (3.5-5.5 g/dL)	3.3 (2.9-3.7)	3.3 (2.9-3.7)	3.3 (2.9-3.7)	3.2 (2.7-3.4)	.609		0.328	0.158-0.653	.002

Kruskal–Wallis test and univariate logistic regression (mortality) were performed for all variables.
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; CRP, C-reactive protein; fT3, free triiodothyronine; fT4, free thyroxine; IL-6, interleukin 6; IQR, interquartile range; LDH, lactate dehydrogenase; N/L ratio, neutrophil–lymphocyte ratio; OR, odds ratio; rT3, reverse triiodothyronine; TSH, thyrotropin.

and used the following strategy: "rs225014" or rs225014-T/C or "thr92ala" or "dio2 a/g" or "T92A." Only studies observing these 3 inclusion criteria were selected: (1) observational studies (cohort, case–control, and cross-sectional studies) on the Thr92Ala-DIO2 polymorphism, (2) studies that included patients with diseases nonrelated to thyroid dysfunction, and (3) presence of control group. Data were independently extracted by 2 authors (F.E.L.B., G.C.) using a predetermined structured form. Disagreements were solved through discussion or when needed by consulting a third author (H.E.R.).

Statistics

The strength of the association between Thr92Ala-DIO2 polymorphism and diseases was measured by OR with 95% CI for dominant (Thr/Thr vs Thr/Ala + Ala/Ala), recessive (Ala/Ala vs Thr/Ala + Thr/Thr), and overdominant (Thr/Ala vs Thr/Thr + Ala/Ala). The chi-squared–based Q-test was used to assess the between-study heterogeneity and $P = .1$ was considered to indicate significant heterogeneity among studies. I^2 statistic and Cochran Q test were applied to examine the heterogeneity and the pooled OR estimation of each study was calculated by the fixed and random-effects model (the Mantel–Haenszel method). The I^2 statistic was specifically documented for the percentage of study variability observed due to heterogeneity rather than chance (I^2 = 0-25%, no heterogeneity; I^2 = 25-50%, moderate heterogeneity; I^2 = 50-75%, high heterogeneity; I^2 = 75-100%, extreme heterogeneity) (48).

All statistical tests were performed using Review Manager 5.4 (The Cochrane Collaboration, UK). $P < .05$ was considered statistically significant.

Results

Patient Demographics and Clinical Characteristics

A total of 274 consecutive patients admitted with COVID-19 were evaluated for potential enrollment in the study. In total, 245 patients met the inclusion criteria and were considered as potential subjects for the study. An additional 25 patients were excluded for lack of genotype determination. The remaining 220 patients completed the study (Fig. 1). The median age was 62 (48-74) years, and 135 patients (61.3%) were male. Baseline sociodemographic and clinical characteristics are summarized in Table 1. Most patients had underlying diseases, including hypertension (65.4%), diabetes (42.3%), and cardiopathy (12.7%), neoplasia (0.9%), and chronic pneumopathy (4.5%). The group of 220 patients was stratified into 3 subgroups: Thr/Thr (n = 79), Thr/Ala (n = 119) and Ala/Ala (n = 23) (Fig. 1). The Thr allele frequency was 0.62 and the Ala allele frequency was 0.37, with a distribution that was in Hardy–Weinberg equilibrium ($P = .07$; chi-squared test).

Blood biochemistry is shown in Table 2. Several thyroid function tests and markers of inflammation, tissue damage, or hemochromocytometric parameters were found to predict mortality using the univariate logistic regression (Table 2). Most parameters were not affected by the patient's genotype except for fT3, ALT, and hemoglobin levels (Table 2).

Clinical Outcomes

Primary

The overall mortality, regardless of genotype, was 38 (17.3%) (Fig. 2). Mortality was lower in Ala/Thr patients (12.6%) than in Thr/Thr patients (21.7%) or Ala/Ala patients (23%) (Fig. 2). Logistic regression analysis confirmed that the presence of the Thr/Ala allele was associated with reduced mortality when compared with Thr/Thr, even after correcting for 14 comorbidities and other covariates (Table 3).

The Cochran–Armitage test was used to assess the relationship between genotype and mortality according to 4 inheritance models. The dominant, recessive, and codominant models did not predict a relationship, although the last one revealed a borderline statistical significance for the heterozygous group (Fig. 2). At the same time, an association between lower mortality and Thr92Ala-DIO2 heterozygosity was statistically significant when the overdominant model was utilized. This is illustrated in the Kaplan–Meier survival analysis (Fig. 3). Logistic regression analysis confirmed that the presence of the

	Genotype	Non survivor (n)	Mortality (%)	Qui-square or Cochran-Armitage test	OR (95% CI)*	P value
Overall	TT + TC + CC	38/220	17.3%	-	-	-
Inheritance models						
Codominant	TT	18/78	23.1%	$\chi^2 = 3.75$, P = 0.05	1	-
	TC	15/119	12.6%		0.48	0.06
	CC	5/23	21.7%		1.06	0.91
Dominant	TT	18/78	23.1%	$\chi^2 = 2.84$, P = 0.09	1.80 (0.88 – 3.65)	0.1
	CC + TC	20/142	14.1%		0.55 (0.27 – 1.13)	-
Recessive	TC + TT	33/197	16.8%	$\chi^2 = 0.35$, P = 0.54	0.72 (0.26 – 2.30)	0.54
	CC	5/23	21.7%		1.38 (0.43 – 3.76)	-
Overdominant	TT + CC	23/101	22.8%	$\chi^2 = 3.95$, P = 0.04	2.09 (0.99 – 4.19)	0.05
	TC	15/119	12.6%		0.49 (0.23 – 1.00)	-

0 5 10 15 20 25
Mortality (%)

Figure 2. DIO2 Thr92Ala polymorphism and mortality (Cochran–Armitage test and chi-squared test); CI, confidence interval; OR, odds ratio; X^2, chi squared.

Table 3. Multivariable regression analyses of mortality, considering multiple covariates, Thr92Ala-DIO2 polymorphism, and overdominant inheritance model

				Ala/Thr *vs* Ala/Ala			Ala/Thr *vs* Thr/Thr			Thr/Thr *vs* Ala/Ala			Ala/Thr *vs* Ala/Ala + Thr/Thr (Overdominant model)		
				OR*	CI 95%	*P*	OR*	CI 95%	*P*	OR*	CI 95%	*P*	OR*	CI 95%	*P*
			Mortality	0.52	0.18-1.75	.26	0.49	0.23-1.04	0.06	1.06	0.36-3.57	.9	0.52	0.26-1.04	0.07
Model 5	Model 4	Model 1	Male gender	0.52	0.18-1.76	.26	0.49	0.23-1.04	0.06	1.06	0.36-3.57	.9	0.52	0.26–1.04	0.07
			Age > 60 year	0.53	0.18-1.79	.27	0.50	0.23-1.06	0.07	1.07	0.36-3.59	.9	0.53	0.26–1.06	0.07
			Diabetes	0.48	0.16-1.62	.20	0.45	0.21-0.98	0.04	1.05	0.36-3.53	.9	0.49	0.23–0.98	0.047
			Hypertension	0.52	0.18-1.75	.26	0.49	0.23-1.06	0.07	1.05	0.36-3.55	.9	0.53	0.26–1.06	0.08
			Hospital stay	0.47	0.15-1.65	.21	0.48	0.21-1.07	0.07	0.97	0.32-3.39	1.0	0.51	0.24–1.05	0.07
			Model 1	0.43	0.14-1.54	.17	0.43	0.19-0.99	0.048	0.99	0.32-3.51	1.0	0.46	0.21–0.98	0.046
		Model 2	TSH	0.51	0.17-1.72	.24	0.49	0.23-1.03	0.06	1.04	0.35-3.51	.9	0.52	0.26–1.05	0.07
			Free T3	0.74	0.24-2.65	.62	0.54	0.25-1.16	0.12	1.37	0.44-4.87	.6	0.62	0.3–1.28	0.20
			Free T4	0.51	0.17-1.74	.25	0.49	0.23-1.03	0.06	1.06	0.36-3.55	.9	0.52	0.25–1.04	0.07
			Reverse T3	0.48	0.16-1.66	.22	0.54	0.25-1.17	0.12	0.89	0.29-3.07	.8	0.56	0.27–1.13	0.11
			Model 2	0.61	0.19-2.24	.42	0.56	0.25-1.22	0.14	0.92	0.25-1.22	.9	0.64	0.3–1.32	0.23
		Model 3	IL-6	0.51	0.17-1.73	.25	0.49	0.23-1.04	0.06	1.05	0.26-3.52	.9	0.52	0.25–1.04	0.07
			CRP	0.55	0.16-2.19	.36	0.41	0.18-0.9	0.03	1.34	0.4-5.36	.6	0.46	0.21–0.96	0.04
			D-DIMER	0.57	0.19-1.97	.34	0.51	0.23-1.11	0.09	1.12	0.37-3.85	.9	0.55	0.27–1.13	0.11
			Neutrophil	0.47	0.16-1.61	.20	0.44	0.2-0.96	0.04	1.06	0.36-3.59	.9	0.48	0.23–0.97	0.04
			LDH	0.46	0.15-1.58	.19	0.47	0.21-1.05	0.07	0.97	0.32-3.33	1.0	0.49	0.23–1.03	0.06
			Model 3	0.52	0.14-2.2	.34	0.36	0.15-0.85	0.02	1.44	0.41-6.08	.6	0.42	0.18–0.92	0.03
			Model 4	0.46	0.12-2.04	.27	0.32	0.12-0.8	0.02	1.43	0.38-6.41	.6	0.38	0.16–0.87	0.03
			Model 5	0.56	0.13-2.85	.46	0.32	0.12-0.85	0.03	1.74	0.4-9.02	.5	0.40	0.16–0.98	0.049

Multivariable regression analyses: Model 1, adjusted for male gender, age >60 years, diabetes, arterial hypertension, and length of hospital stay. Model 2, adjusted for thyrotropin, free triiodothyronine, free thyroxine, and reverse triiodothyronine. Model 3, adjusted for interleukin-6, neutrophils, lactate dehydrogenase, C-reactive protein, and D-dimer. Model 4, adjusted for Model 1 and 3. Model 5, adjusted for all of the above variables.

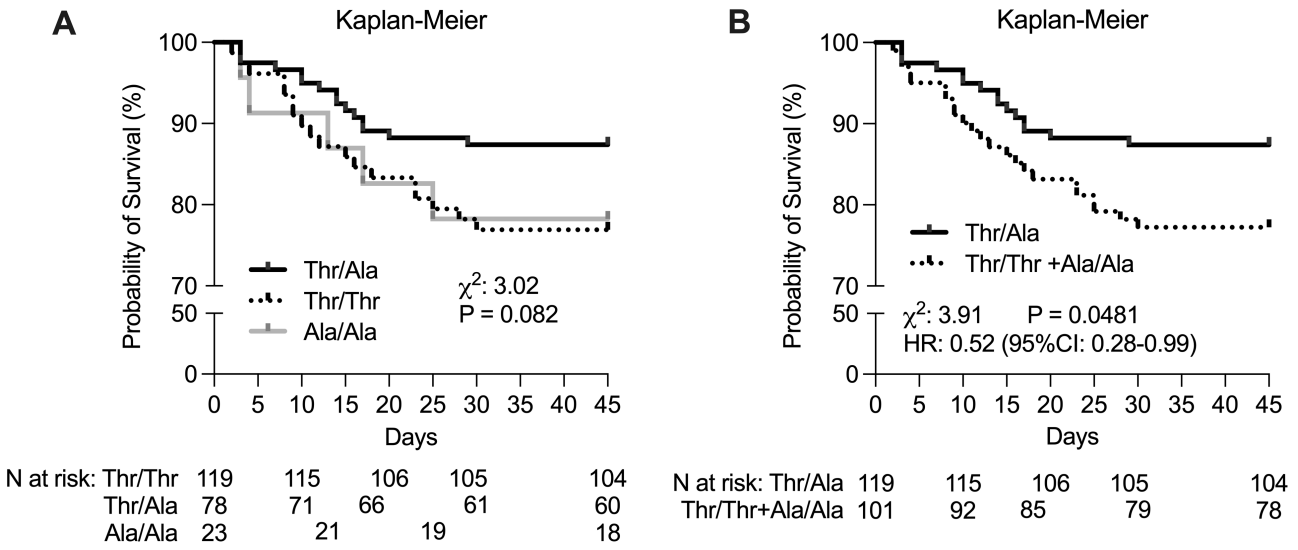

Figure 3. Kaplan–Meier survival curve and their association with mortality. Kaplan–Meier survival curves of DIO2 Thr92Ala polymorphism for the overall survival in patients with COVID-19. (Thr/Thr vs Thr/Ala vs Ala/Ala and Thr/Ala vs Thr/Thr + Ala/Ala). IQR, interquartile range; HR, hazard ratio.

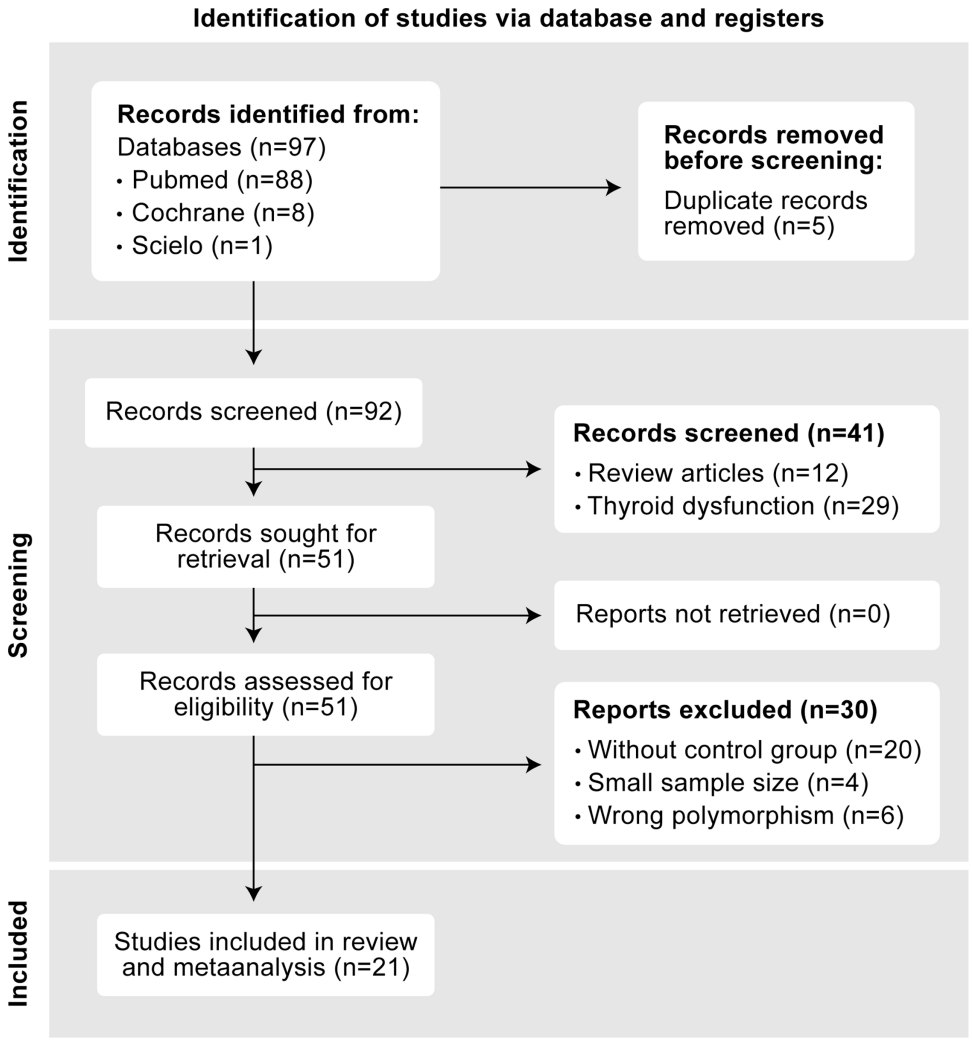

Figure 4. Flow diagram utilized in metanalysis (PRISMA 2020).

Table 4. Fixed and random-effects meta-analysis of the haplotypic association between the occurrence of a series of diseases and the Thr92Ala-DIO2 polymorphism according to 3 inheritance models: dominant, overdominant and recessive

			Dominant model		Overdominant		Recessive	
			(Thr/Thr vs Thr/Ala + Ala/Ala)		(Thr/Ala vs Thr/Thr + Ala/Ala)		(Ala/Ala vs Thr/Ala + Thr/Thr)	
Author, year	N	Major endpoint	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)
1. Marcondes, 2021	132	Autism	.4	1.41 (0.62-3.19)	.6	0.84 (0.40-1.75)	.7	0.86 (0.37-1.99)
2. Kang, 2020	6022	Osteoporosis	.4	0.82 (0.50-1.34)	.7	0.90 (0.58-1.41)	.1	1.48 (0.89-2.48)
3. Galecka, 2015	331	Recurrent depressive disorder	.5	1.16 (0.75-1.79)	.6	1.13 (0.73-1.74)	.01	0.07 (0.01-0.56)
4. Lee, 2015	809	Alcohol dependence	.7	0.94 (0.70-1.27)	1.0	0.99 (0.74-1.32)	.6	1.12 (0.76-1.63)
5. Luo, 2015	260	Mild cognitive impairment	.8	0.92 (0.55-1.55)	.9	0.97(0.59-1.58)	.6	1.16 (0.65-2.06)
6. Huang, 2013	469	Kashin–Beck disease	.8	1.06 (0.71-1.60)	.9	0.98 (0.64-1.50)	.6	0.70 (0.22-2.23)
7. Nair, 2012	300	Diabetes (Pima Indians)	.2	0.43 (0.13-1.43)	.05	0.60 (0.36-1.01)	.01	1.86 (1.13-3.04)
8. Xiong, 2010	370	Kashin-Beck disease	.9	0.96 (0.61-1.51)	.7	0.91 (0.61-1.38)	.5	1.21 (0.73-2.02)
9. Dora, 2010	1573	Diabetes	.5	0.93 (0.75-1.15)	.3	0.90 (0.73-1.11)	.02	1.43 (1.05-1.96)
10. He, 2009	558	Bipolar disorder	.009	0.61 (0.42-0.88)	.4	0.87 (0.62-1.21)	.0001	2.27 (1.50-3.44)
11. Meulenbelt, 2008	3114	Osteoarthritis	.9	1.01 (0.86-1.18)	.002	0.79 (0.67-0.91)	<.0001	1.64 (1.32-2.04)
12. Van der Deure, 2008	2294	Hypertension	.7	1.04 (0.88 -1.23)	.6	1.04 (0.88-1.23)	.2	0.84 (0.66-1.07)
13. Maia, 2007	1631	Diabetes	.7	1.06 (0.77-1.47)	.4	0.86 (0.63-1.19)	.4	1.22 (0.78-1.92)
14. Gumieniak, 2007	372	Hypertension	.007	0.48 (0.28-0.82)	.004	2.30 (1.31-4.03)	.7	0.85(0.41-1.76)
15. Grarup, 2006	6175	Diabetes	.3	1.06 (0.94-1.20)	.1	0.91 (0.81-1.03)	.4	1.08 (0.90-1.28)
16. Grarup, 2006	5843	Obesity	.1	1.11 (0.97-1.28)	.8	0.98 (0.86-1.13)	.1	0.82 (0.66-1.02)
17. Mentuccia, 2005	1268	Diabetes	.5	0.91 (0.66-1.24)	.7	0.95 (0.65-1.30)	.1	1.52 (0.93-2.49)
18. Guo, 2004	538	Mental retardation	.7	1.07 (0.75-1.52)	.6	0.69 (0.48-0.99)	.9	1.03 (0.65-1.65)
Metanalysis total (fixed)	27 503		.7	1.01 (0.95-1.07)	.01	0.93 (0.88-0.98)	.001	1.15 (1.06-1.24)
Metanalysis total (random)	27 503		.7	0.99 (0.92-1.07)	.05	0.93 (0.87-1.00)	.04	1.19 (1.01-1.41)
Heterogeneity analysis				Tau^2 = .01; chi^2 = 22.43, *P* = .17; I^2 = 24%		Tau^2 = 0.00; chi^2 = 21.64, *P* = 0.20; I^2 = 21%		Tau^2 = .07; chi^2 = 53.73, *P* < .0001; I^2 = 68%
Number of studies with $P \leq .05$				3 studies		3 studies		5 studies
Advantage (n)/disadvantage (n)				2/1		2/1		1/4

Thr/Ala allele was associated with reduced mortality when compared with Thr/Thr and Ala/Ala, even after accounting for comorbidities (Table 3).

Secondary

As a group, the median hospital stay was 6 (4-10) days. The severity score systems indicated that patients were moderately/gravely affected by the disease, with developments such as admission to ICU (25%), cardiovascular shock (12.7%), and/or endotracheal intubation (13.2%). As expected, length of stay, ICU admission, cardiovascular shock, and endotracheal intubation correlated with mortality as assessed through univariate logistic regression analysis (Table 1). The analyses of these parameters in the 3 genotype subgroups, namely Thr/Thr, Ala/Thr, Ala/Ala, did not reveal significant differences except for hypertension as an underlying condition, which was higher in the Thr/Thr group (Table 1).

Meta-analysis

The results of the systematic search and selection led us to 97 studies, which after application of the inclusion and exclusion criteria, were reduced to 21 studies totaling 8400 cases and 20 165 controls (Fig. 4). The analysis focused on 2 major types of studies: (1) those that reported the frequency of the 2 different alleles (Ala and Thr) for each disease and (2) those that reported clinical outcomes for each disease as a function of these 2 different alleles.

In the frequency analysis, the reported allele frequency was analyzed according to the 3 inheritance models, dominant, overdominant, and recessive. The summary of the resulting fixed and random-effects meta-analysis for the haplotypic association of DIO2 SNPs (Thr/Thr, Thr/Ala, and Ala/Ala) with the different diseases, as well as heterogeneity test, are shown in Table 4 and Fig. 5. The application of the recessive model revealed that the Ala/Ala genotype was associated with a greater risk of having any of the diseases (random OR 1.19 [1.01-1.41], $P = .04$). At the same time, the application of the overdominant model revealed that carrying the Thr/Ala genotype was associated with a smaller risk of having the diseases (random OR 0.93 [0.87-1.0], $P = .05$). No significant associations were observed when the dominant model was applied. Notably, both the Q statistical test (chi squared = 53.7, $P < .0001$) and the I^2 statistic ($I^2 = 68\%$) showed high heterogeneity for the recessive model, whereas the dominant (chi squared = 22.4, $P = .17$, $I^2 = 24\%$) and overdominant models exhibited no heterogeneity (chi squared = 21.6, $P = .20$, $I^2 = 21\%$) (Table 4 and Fig. 5).

A similar analysis was performed while considering the clinical outcomes. The reported outcome was also analyzed according to the 3 inheritance models. The summary of the resulting fixed and random-effects meta-analysis for the haplotypic association of DIO2 SNPs with the different outcomes, as well as heterogeneity test, are shown in Table 5 and Fig. 6. The dominant inheritance model revealed that carrying the Thr/Thr genotype was associated with a worse outcome (fixed OR 2.18 [1.43-3.3], $P = .0003$). At the same time, the overdominant model revealed an inverse association between the Thr/Ala genotype and the severity of the clinical outcome (fixed OR 0.54 [0.40-0.75], $P = .0002$). No significant associations between genotype and clinical outcomes were observed after using the recessive inheritance model (fixed OR 1.27 [0.88-1.83], $P = .20$) (Table 5 and Fig. 6).

Discussion

To our knowledge, this is the first prospective study of patients hospitalized with COVID-19 in which the primary clinical outcome (mortality) was analyzed according to the Thr92Ala-DIO2 polymorphism. Our findings revealed that carrying the Thr/Ala genotype was associated with lower mortality rates. The Kaplan–Meier curve shows that the heterozygous genotype (Thr/Ala) was associated with a 47% reduced risk of intrahospital mortality (Fig. 3). The univariate and multivariate logistic regression, adjusted for multiple

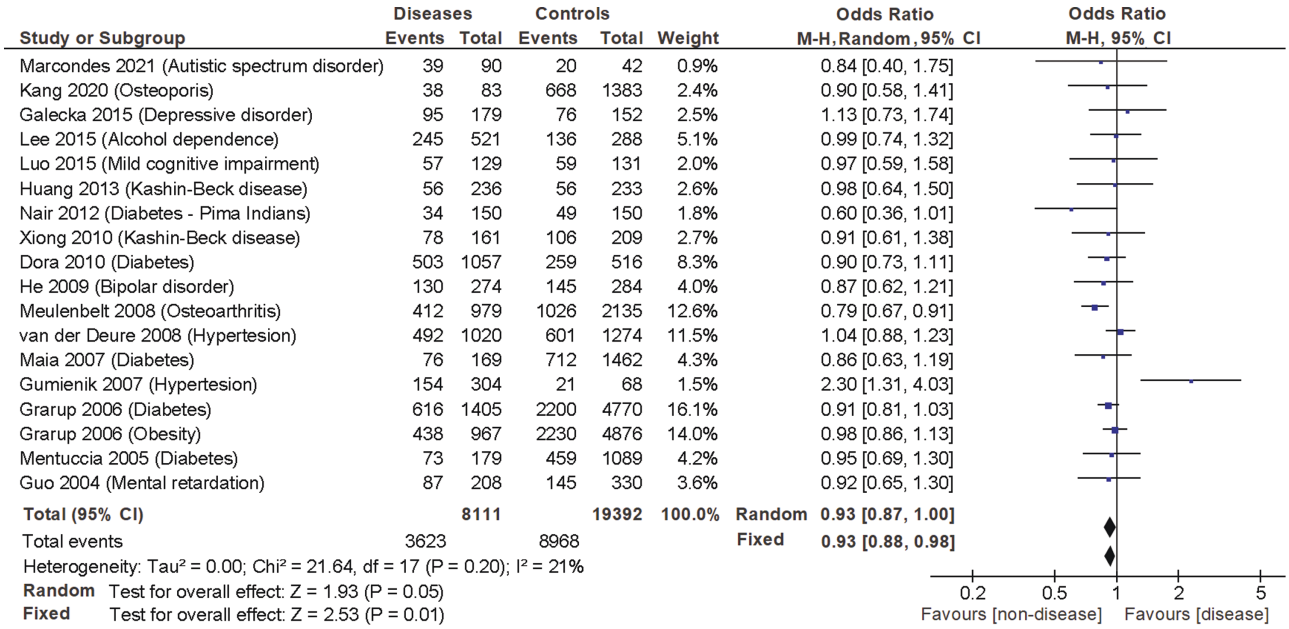

Figure 5. Fixed and random-effects meta-analysis of the haplotypic association between the occurrence of a series of diseases and the Thr92Ala-DIO2 polymorphism according to overdominant inheritance models.

covariates, indicated a reduction in mortality that ranged from 51% to 66% (Table 3).

These results were unexpected and prompted us to examine other studies that correlated the Thr92Ala-DIO2 polymorphism with other clinical outcomes. The metanalysis of 5 studies (including the present study) involving 1062 patients revealed that carriers of the Thr/Ala genotype exhibited significantly better clinical outcomes. In addition, the metanalysis of 16 studies involving 27 503 patients revealed that carriers of the Thr/Ala genotype were less likely to be found among patients with 12 medical conditions. These results shed light on a previously unappreciated aspect of the Thr92Ala-DIO2 polymorphism, which is a likely advantage for carriers of the heterozygous genotype. Such a condition, in which the phenotype of the heterozygote lies outside the phenotypical range of both homozygous, is known as overdominance.

Overdominance has been described for other conditions as well. For example, there is evidence that genetic heterozygosity in humans provides greater resistance to certain viral infections. A study that evaluated human leukocyte antigen (HLA) polymorphisms in patients with HIV revealed that heterozygosity of 1 or more loci was associated with a slower progression to AIDS and reduced mortality (49). Heterozygosity advantage of human leukocyte antigen polymorphisms has also been reported for hepatitis B virus and hepatitis C infections (50, 51).

Sequencing DIO2 from archaic human subspecies led some to conclude that Neanderthals and Denisovans displayed only the Ala92-DIO2 allele, suggesting that those hominines were homozygous for Ala92-DIO2 (52). The fact that Ala92-D2 is about 20% less catalytically active (29) suggests that the minor allele could have protected against iodine deficiency because it metabolizes less T4. The Thr92-DIO2 appeared for the first time during the Upper Pleistocene and has been conserved during the Neolithic age. The fact that the Thr92-DIO2 allele became the major one observed in the modern human suggests that its existence confers an evolutionary advantage.

The present studies suggest that such an evolutionary advantage is identified by the increased survival of Thr/Ala patients during admission for COVID-19. In fact, the metanalyses suggest that this protective effect might be broader and include multiple other chronic conditions and severe diseases, such as ischemic stroke, myocardial infarction, and left ventricular hypertrophy. While the strength of these associations needs to be confirmed in other settings and with much larger populations, one can only speculate as to the mechanistic explanation involved. COVID-19-induced respiratory failure promotes angiocentric inflammation, characterized by generalized thrombosis with microangiopathy, vasoconstriction, and distinct intussusceptive angiogenesis (53). A series of genes involved in these pathogenetic mechanisms of COVID-19 have been recently identified, including *CXCR4* (53, 54) and *SLC44A2* (55-57). Genes involved in the activation of the immune response and secretion of cytokines, such as CDK2 (58), BST2 (59), and CCL4 (60-62), have also been identified. In the specific case of lung inflammatory diseases, a study in mice found that endoplasmic reticulum stress may play a central role. Moreover, inhibition of endoplasmic reticulum stress alleviated endotoxin-induced ALI both in vivo and in vitro (63, 64). This is relevant given that expressing the Thr92Ala-DIO2 gene has been linked to endoplasmic reticulum stress in cell and animal models (29).

Table 5. Fixed and random-effects meta-analysis of the haplotypic association between disease outcome and the Thr92Ala-DIO2 polymorphism according to 3 inheritance models: dominant, overdominant and recessive

			Dominant model		Overdominant		Recessive	
			(Thr/Thr vs Thr/Ala + Ala/Ala)		(Thr/Ala vs Thr/Thr + Ala/Ala)		(Ala/Ala vs Thr/Ala + Thr/Thr)	
Author, year	N	Major endpoint	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)
1. Beltrao, 2021	220	COVID-19 death	.1	1.83 (0.90-3.71)	.05	0.49 (0.24-1.00)	.6	1.38 (0.48-3.98)
2. Taroza, 2020	168	Depression after AIS	.3	2.20 (0.47-10.2)	.4	0.72 (0.36-1.43)	.6	1.21 (0.61-2.40)
3. Kazukauskiene, 2020	283	Cardiac-related death in ICU	.1	2.52 (0.77-8.23)	.3	0.54 (0.16-1.75)	.6	0.42 (0.02-7.29)
4. Taroza, 2019	248	AIS severity	.4	1.53 (0.52-4.49)	.04	0.58 (0.35-0.98)	.1	1.54 (0.92-2.57)
5. Grineva, 2009	143	LVH with Graves's disease	.007	3.06 (1.36-6.90)	.02	0.36 (0.15-0.87)	.4	0.49 (0.10-2.34)
Metanalysis total (Fixed)	1062		.0003	2.18 (1.43-3.3)	.0002	0.54 (0.40-0.75)	.20	1.27 (0.88-1.83)
Metanalysis total (Random)	1062		.0004	2.17 (1.41-3.3)	.0002	0.55 (0.40-0.75)	.17	1.30 (0.90-1.88)
Heterogeneity analysis			$Tau^2 = 0.00$; $chi^2 = 1.37$, $P = .85$; $I^2 = 0\%$		$Tau^2 = 0.00$; $chi^2 = 1.57$, $P = 0.81$; $I^2 = 0\%$		$Tau^2 = 0.00$; $chi^2 = 2.57$, $P = .63$; $I^2 = 0\%$	
Number of studies with $P \leq .05$			1 studies		3 studies		0 studies	
Advantage (n)/Disadvantage (n)			0/1		3/0		0/0	

Abbreviations: AIS, acute ischemic stroke; ICU, intensive care unit; LVH, left ventricular hypertrophy.

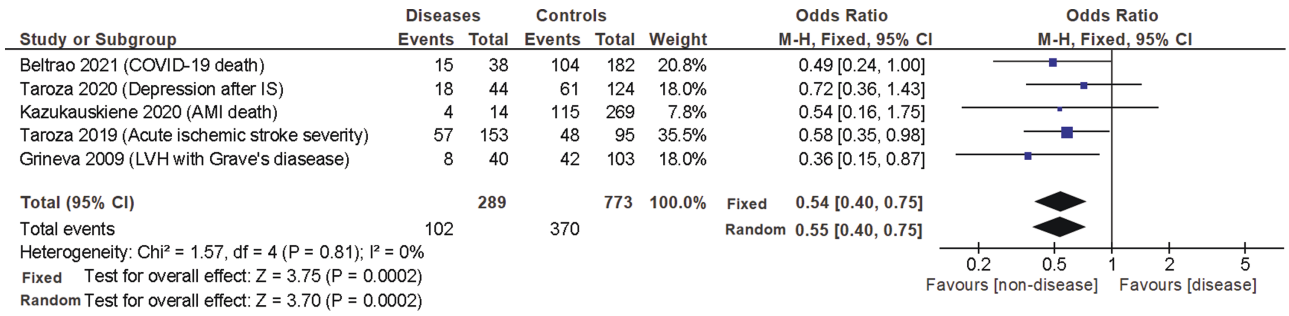

Figure 6. Fixed and random-effects meta-analysis of the haplotypic association between disease outcome and the Thr92Ala-DIO2 polymorphism according to overdominant inheritance models. AMI, acute myocardial infarction; IS, ischemic stroke; CI, confidence interval; ICU, intensive care unit; LVH, left ventricular hypertrophy; M-H, Mantel–Haenszel.

While studying post-mortem samples of the human temporal lobe and HEK-293 cells stably expressing Ala92-DIO2, it was observed in both models that the expression of different DIO2 alleles correlates with the expression of 81 genes related to inflammation, oxidative stress, apoptosis, mitochondrial dysfunction, DNA repair, growth factor signaling, and neurodegenerative diseases (65). Remarkably, the Thr/Ala genotype exhibited a strong positive correlation with the expression of CXCR4, SLC44a2 in both cells and human brain samples; the Thr/Thr genotype correlated positively with the expression of CDK2, BST2; and Ala/Ala genotype correlated positively with CXCR4, SLC44a2, and BST2.

The present study is limited by the lack of a conclusive mechanistic explanation for the protective role played by the Thr92Ala-DIO2 heterozygosity. The study is also limited by the relatively small number of patients, which has an effect size index of 0.3 (best if below 0.2), and by the fact that we only considered patients hospitalized with COVID-19 with moderate to severe conditions. Individuals with mild disease were not evaluated.

Conclusion

Here we provide evidence for a protective role played by the Thr92Ala-DIO2 heterozygosity in patients with COVID-19. An accompanying metanalysis suggests that this advantage is extended to other conditions.

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Disclosure Summary

A.B. is a consultant for AbbVie, Synthonics, and Allergan. The other authors declare no relevant disclosures.

Data Availability

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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