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

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Disclosures

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Abbreviations: AIDS: acquired immunodeficiency syndrome, ALI: acute lung injury, ALT: Alanine transaminase, AMI: acute myocardial infarction, anti-TPO: anti-thyroid peroxidase antibodies, ARDS: Acute respiratory distress syndrome, AST: aspartate aminotransferase, 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, C-X-C chemokine receptor type 4: CXCR4, DIO2: Type II deiodinase, DIO3: Type III deiodinase, ED: emergency department, ER: endoplasmic reticulum, fT3: free triiodothyronine, fT4: free thyroxine, HLA: human leukocyte antigen, HR: hazard ratio, ICU: Intensive Care Unit, IL-6: interleukin 6, IQR: Interquartile range, IS: ischemic stroke, LDH: Lactate dehydrogenase, LVH: left ventricular hypertrophy, M-H: Mantel Haenszel, NEWS2: National Early Warning Score 2, NLR: neutrophil-lymphocyte ratio, NTIS: nonthyroidal illness syndrome, OR: Odds ratio, qSOFA: Quick Sepsis-related Organ Failure Assessment, rRT-PCR: real-time reverse-transcriptase-polymerase chain reaction,
SARS: severe acute respiratory syndrome, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SNP: single nucleotide polymorphisms, SLC44a2: Solute carrier family 44 member 2; TH: thyroid hormones, fT₃: free triiodothyronine, TSH: thyroid-stimulating hormone.
Abstract

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

Methods: Here we conducted an observational, longitudinal, and prospective cohort study to investigate a possible association between the Thr92Ala-DIO2 polymorphism and intra-hospital 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: 220 consecutive patients [median age: 62 (48–74) years] were stratified into three 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%) when compared to Thr/Thr patients (21.7%) or Ala/Ala patients (23%). The heterozygous genotype (Thr/Ala) was associated with a 47% reduced risk of intra-hospital mortality whereas the univariate and multivariate logistic regression adjusted for multiple covariates, revealed a reduction that ranged from 51-66%. The association of the Thr/Ala genotype with better clinical outcomes was confirmed in a metaanalysis of 5 studies, including the present one.

Conclusion: Here we provide evidence for a protective role played by the 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.

Keywords: Thyroid, Type II deiodinase, Polymorphism and COVID-19.
Introduction

The Coronavirus 2019 (COVID-19) pandemic, caused by the 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. SARS-Cov-2 infection can cause pulmonary and systemic inflammation, leading to multiple organ dysfunction in high-risk populations such as the elderly, pregnant women, patients with obesity, hypertension, diabetes, and those on long-term immunosuppressive therapy. The lethality among hospitalized patients ranges from 11% and 15%.

The most feared complication of COVID-19 is the severe acute respiratory syndrome (SARS) which has an in-hospital prevalence of 17 to 29%. Acute lung injury (ALI) is one 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. In this regard, it is well accepted that the low T3 syndrome is a strong predictor of poor prognosis in critically ill hospitalized patients, including patients admitted to the intensive care unit, pneumonia, severe burns, stroke, septic shock, respiratory failure, cardiovascular diseases, multiple trauma. This has also been found in patients with COVID-19, with strong association between reduced levels of free T3 (fT3) at the admission of hospitalized patients with intra-hospital severity and mortality.

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 thyroid hormone (TH) T3 increased alveolar fluid clearance in rat lungs with hypoxia-induced lung injury. 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.
Accordingly, Ma et al. (2011) 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 thyroid hormone metabolism is intimately linked to the integrated response to inflammatory injury in critically ill patients.  

DIO2 is located on the long arm of chromosome 14, at position 14q24.3. To date, six single nucleotide polymorphisms (SNPs) of these genes have been described in the literature, including rs225014, ORFa-Gly3Asp, rs225010, rs225012, rs2267872, and rs1388378. The rs225014 SNP, also known as 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. Nonetheless, carrying the Thr92Ala-DIO2 polymorphism does not affect the thyroid function tests in individuals that have a normal thyroid gland but this is controversial in thyroidectomized patients kept on LT4. In a large report that included approximately 550 LT4-treated patients, the Thr92Ala-DIO2 polymorphism had no effect on the serum T4:T3 ratio, but in another series of 140 patients it was associated with a higher T4:T3 ratio.

The minor allele Ala92-Dio2 has a high prevalence in the population (38.8 to 47.6%). Several studies have analysed its association with multiple chronic diseases, such as type 2 diabetes mellitus, obesity, arterial hypertension, osteoarthritis, osteoporosis, and dementias, many of which are risk factors for a worse prognosis for COVID-19, but controversy has prevented the formulation of a unifying hypothesis as to its role. Here we tested whether the Thr92Ala-DIO2 polymorphism is associated with intra-hospital 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 (ED) between June-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

220 consecutive patients with a positive result on standard of care reverse transcriptase polymerase chain reaction test (RT-qPCR - Biomol OneStep/COVID-19, IBMP, Paraná, Brazil) for SARS-CoV-2 in nasopharyngeal swab, or, in cases of negative RT-qPCR, having clinical, radiological, and serological (IgG positive for SARS-CoV-2) criteria. Samples were centrifuged at 2,000 g for 15 min at 4 °C and subsequently frozen at −80 °C until measurement. Exclusion criteria were: (i) patient younger than 18 years, (ii) patient with a history of thyroid disease and diagnosis of pregnancy, and (iii) patient who used iodinated contrast in the last six 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 48h of admission, patient severity was first defined using three scoring systems: (i) the quick Sepsis-related Organ Failure Assessment (qSOFA), the National Early Warning Score 2 (NEW2), and chest computed tomography (CT) severity score proposed by Pan et al. 45.
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 cytokines 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), thyroid-stimulating hormone (TSH)], thyroglobulin, anti-thyroid 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 (NLR) 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 analyzes, a 5 µl solution will be used, containing: 20 ng of sample DNA, 2.5 µl of universal Taqman PCR Master Mix (with final concentration 1X), 0.25 µl of the assay (probe and primers) for the concentration of 20X and 0.125 µl for the concentration of 40X (maintaining a final concentration of 1X), 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 ten 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**: Cumulative mortality during admission according to Thr92Ala-DIO2 polymorphism.

**Secondary**: Blood biochemistry, thyroid function tests, length of stay, comorbidities, complications, 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 (α<0.05) 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-square, or Cochran-Armitage test were used for non-parametric 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<0.05 was accepted as statistically significant. The statistical program GraphPad Prism, v.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) versus 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 - IL6, 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 and used the following strategy: ‘rs225014’ or rs225014-T/C or ‘thr92ala’ or ‘dio2 a/g’ or ‘T92A’. Only studies observing these three inclusion criteria were selected: i) observational studies (cohort, case–control, and cross-sectional studies) on the Thr92Ala-DIO2 polymorphism, ii) studies that included patients with diseases non-related to thyroid dysfunction, and iii) presence of control group. Data were independently extracted by two authors (FELB, GC) using a pre-determined structured form. Disagreements were solved through discussion or when needed by consulting a third author (HER).

Statistics: The strength of the association between Thr92Ala-DIO2 polymorphism and diseases was measured by odds ratio (OR) with 95% confidence intervals (95% CIs) 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 square-based Q-test was used to assess the between-study heterogeneity and a P-value of 0.1 was considered to indicate significant heterogeneity among studies. I² 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 I² statistic was specifically documented for the percentage of study variability observed due to heterogeneity rather than chance (I² = 0–25%, no heterogeneity; I² = 25–50%, moderate heterogeneity; I² = 50–75%, high heterogeneity; I² = 75–100%, extreme heterogeneity) 48.
All statistical tests were performed using Review Manager 5.4 (The Cochrane Collaboration, UK). A P value <0.05 was considered statistically significant.

Results

Patient Demographics and Clinical Characteristics

274 consecutive patients admitted with COVID-19 were evaluated for potential enrollment in the study. 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 three 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=0.07 (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%) when compared to 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 to 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 four inheritance models. The dominant, recessive, and codominant models did not predict a relationship, although the latter 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 over-dominant model was utilized. This is illustrated in the Kaplan-Meier survival analysis (Fig. 3). Logistic regression analysis confirmed that the presence of the Thr/Ala allele was associated with reduced mortality when compared to 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 intensive care unit (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 three genotype subgroups, i.e., 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 20165 controls (Fig. 4). The analysis focused on two major types of studies: (i) those that reported the frequency of the two different alleles (Ala and Thr) for each disease and (ii) those that reported clinical outcomes for each disease as a function of these two different alleles.
In the frequency analysis, the reported allele frequency was analyzed according to the three 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 Figure 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=0.04). At the same time, the application of the over-dominant 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=0.05). No significant associations were observed when the dominant model was applied. Notably, both the Q statistical test (Chi² = 53.7, P <0.0001) and the I² statistic (I² = 68%) showed high heterogeneity for the recessive model, whereas the dominant (Chi² = 22.4, P =0.17, I²=24%) and over-dominant models exhibited no heterogeneity (Chi² = 21.6, P =0.20, I²=21%) (Table 4, Figure 5).

A similar analysis was performed while considering the clinical outcomes. The reported outcome was also analyzed according to the three 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 Figure 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=0.0003). At the same time, the over-dominant 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=0.0002). 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=0.20) (Table 5 and Figure 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 intra-hospital mortality (Figure 3). The univariate and multivariate logistic regression, adjusted for multiple covariates, indicated a reduction in mortality that ranged from 51-66% (Table 3).

These results were unexpected and prompted us to examine other studies that correlated the Thr92Ala-DIO2 polymorphism with other clinical outcomes. The metaanalysis 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 metaanalysis of 16 studies involving 27503 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 one or more loci was associated with a slower progression to acquired immunodeficiency syndrome (AIDS) and reduced mortality. Heterozygosity advantage of HLA polymorphisms has also been reported for hepatitis B virus and hepatitis C infections.

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. The fact that Ala92-D2 is about 20% less catalytically active 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 infarct, 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. A series of genes involved in these pathogenetic mechanisms of COVID-19 have been recently identified, including CXCR4 and SLC44A2. Genes involved in the activation of the immune response and secretion of cytokines, such as CDK2, BST2, and CCL4, have also been identified. In the specific case of lung inflammatory diseases, a study in mice found that endoplasmic reticulum (ER) stress may play a central role. Moreover, inhibition of ER stress alleviated endotoxin-induced ALI both in vivo and in vitro. This is relevant given that expressing the Thr92Ala-DIO2 gene has been linked to ER stress in cell and animal models.

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. 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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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.
1. Yazdanpanah F, Hamblin MR, Rezaei N. The immune system and COVID-19: Friend or foe? *Life Sciences*. 2020; **256**:117900.

2. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. *The BMJ*. 2020;368:m1091.

3. Giovanetti M, Angeletti S, Benvenuto D, Ciccozzi M. A doubt of multiple introduction of SARS-CoV-2 in Italy: A preliminary overview. *Journal of Medical Virology*. 2020; **92**(9):1634-1636.

4. Guan W jie, Ni Z yi, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine*. 2020; **382**(18):1708-1720.

5. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature*. 2020; **584**(7821):430-436.

6. Kim ES, Chin BS, Kang CK, et al. Clinical Course and Outcomes of Patients with Severe Acute Respiratory Syndrome Coronavirus 2 Infection: a Preliminary Report of the First 28 Patients from the Korean Cohort Study on COVID-19. *J Korean Med Sci*. 2020; **35**(13):e142.

7. Park M, Cook AR, Lim JT, Sun Y, Dickens BL. A Systematic Review of COVID-19 Epidemiology Based on Current Evidence. *J Clin Med*. 2020; **9**(4):967.

8. Hudson LD, Milberg JA, Anardi D, Maunder RJ. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 1995; **151**(2 Pt 1):293-301.

9. Guo J, Hong Y, Wang Z, Li Y. Prognostic Value of Thyroid Hormone FT3 in General Patients Admitted to the Intensive Care Unit. *Biomed Res Int*. 2020; **2020**:6329548.

10. Liu MQ, Chen Z, Chen LX. Endoplasmic reticulum stress: a novel mechanism and therapeutic target for cardiovascular diseases. *Acta Pharmacol Sin*. 2016; **37**(4):425-443.

11. Gangemi EN, Garino F, Berchialla P, et al. Low triiodothyronine state: a predictor of poor prognosis in burn patients. *Burns*. 2008; **34**(6):817-824.

12. Alevizaki M, Synetou M, Xynos K, Pappa T, Vemmos KN. Low triiodothyronine: a strong predictor of outcome in acute stroke patients. *Eur J Clin Investig*. 2007; **37**(8):651-657.

13. Borkowski J, Siemiatkowski A, Wołczyoski S, Czaban SL, Jedynak M. Assessment of the release of thyroid hormones in septic shock -- prognostic significance. *Pol Merkur Lekarski*. 2005; **18**(103):45-48.

14. Scoscia E, Baglioni S, Eslami A, Iervasi G, Monti S, Todisco T. Low triiodothyronine (T3) state: a predictor of outcome in respiratory failure? Results of a clinical pilot study. *Eur J Endocrinol*. 2004; **151**(5):557-560.
15. Iervasi G, Pingitore A, Landi P, et al. Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease. *Circulation*. 2003;107(5):708-713.

16. Schilling JU, Zimmermann T, Albrecht S, Zwipp H, Saeger HD. Low T3 syndrome in multiple trauma patients—a phenomenon or important pathogenetic factor?. *Med Klin*. 1999;94 Suppl 3:66-69.

17. Gao W, Guo W, Guo Y, et al. Thyroid hormone concentrations in severely or critically ill patients with COVID-19. *Journal of Endocrinological Investigation*. Published online 2020. doi:10.1007/s40618-020-01460-w

18. Chen M, Zhou W, Xu W. Thyroid Function Analysis in 50 Patients with COVID-19: A Retrospective Study. *Thyroid*. 2021;31(1):8-11.

19. Zou R, Wu C, Zhang S, et al. Euthyroid Sick Syndrome in Patients With COVID-19. *Front Endocrinol (Lausanne)*. 2020;11:566439.

20. Beltrão FEL, Beltrão DCA, Carvalhal G, et al. Thyroid Hormone Levels During Hospital Admission Inform Disease Severity and Mortality in COVID-19 Patients. *Thyroid*. 2021;31(11):1639-1649.

21. Campi I, Bulgarelli I, Dubini A, et al. The spectrum of thyroid function tests during hospitalization for SARS-COV-2 infection. *Eur J Endocrinol*. 2021;184(5):699-709.

22. Bhargava M, Runyon MR, Smirnov D, et al. Triiodo-L-thyronine rapidly stimulates alveolar fluid clearance in normal and hyperoxia-injured lungs. *Am J Respir Crit Care Med*. 2008;178(5):506-512.

23. Yu G, Tzouvelekis A, Wang R, et al. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. *Nat Med*. 2018;24(1):39-49.

24. Ma SF, Xie L, Pino-Yanes M, et al. Type 2 deiodinase and host responses of sepsis and acute lung injury. *Am J Respir Cell Mol Biol*. 2011;45(6):1203-1211.

25. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest*. 2006;116(10):2571-2579.

26. Guo TW, Zhang FC, Yang MS, et al. Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *J Med Genet*. 2004;41(8):585-590.

27. Zhang K, Xi H, Wang X, et al. A family-based association study of DIO2 and children mental retardation in the Qinba region of China. *J Hum Genet*. 2012;57(1):14-17.

28. Mentuccia D, Proietti-Pannunzi L, Tanner K, et al. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes*. 2002;51(3):880-883.
29. Jo S, Fonseca TL, Bocco BMLC, et al. Type 2 deiodinase polymorphism causes ER stress and hypothyroidism in the brain. *Journal of Clinical Investigation*. 2019;129(1):230.

30. Wouters HJCM, van Loon HCM, van der Klauw MM, et al. No Effect of the Thr92Ala Polymorphism of Deiodinase-2 on Thyroid Hormone Parameters, Health-Related Quality of Life, and Cognitive Functioning in a Large Population-Based Cohort Study. *Thyroid: official journal of the American Thyroid Association*. 2017;27(2):147-155.

31. Panicker V, Saravanan P, Vaidya B, et al. Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *The Journal of clinical endocrinology and metabolism*. 2009;94(5):1623-1629.

32. Castagna MG, Dentice M, Cantara S, et al. DIO2 Thr92Ala Reduces Deiodinase-2 Activity and Serum-T3 Levels in Thyroid-Deficient Patients. *The Journal of clinical endocrinology and metabolism*. 2017;102(5):1623-1630.

33. Dora JM, Machado WE, Rheinheimer J, Crispim D, Maia AL. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *Eur J Endocrinol*. 2010;163(3):427-434.

34. Nair S, Muller YL, Ortega E, Kobes S, Bogardus C, Baier LJ. Association analyses of variants in the DIO2 gene with early-onset type 2 diabetes mellitus in Pima Indians. *Thyroid*. 2012;22(1):80-87.

35. Canani LH, Capp C, Dora JM, et al. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2005;90(6):3472-3478.

36. Grarup N, Andersen MK, Andreasen CH, et al. Studies of the common DIO2 Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish white subjects. *J Clin Endocrinol Metab*. 2007;92(1):363-366.

37. Gumieniak O, Perlstein TS, Williams JS, et al. Ala92 type 2 deiodinase allele increases risk for the development of hypertension. *Hypertension*. 2007;49(3):461-466.

38. van der Deure WM, Peeters RP, Uitterlinden AG, et al. Impact of thyroid function and polymorphisms in the type 2 deiodinase on blood pressure: the Rotterdam Study and the Rotterdam Scan Study. *Clin Endocrinol (Oxf)*. 2009;71(1):137-144.

39. Meulenbelt I, Min JL, Bos S, et al. Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. *Hum Mol Genet*. 2008;17(12):1867-1875.

40. Kang YE, Kang YM, Park B, Shong M, Yi HS. Type 2 deiodinase Thr92Ala polymorphism is associated with a reduction in bone mineral density: A community-based korean genome and epidemiology study. *Clin Endocrinol (Oxf)*. 2020;93(3):238-247.
41. Luo M, Zhou XH, Zou T, Keyim K, Dong LM. Type II deiodinase polymorphisms and serum thyroid hormone levels in patients with mild cognitive impairment. Genet Mol Res. 2015;14(2):5407-5416.

42. Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nat Rev Endocrinol. 2021;17(1):11-30.

43. Friedman AN, Guirguis J, Kapoor R, et al. Obesity, inflammatory and thrombotic markers, and major clinical outcomes in critically ill patients with COVID-19 in the US. Obesity (Silver Spring). 2021;29(10):1719-1730.

44. Ye Q, Wang B, Mao J, et al. Epidemiological analysis of COVID-19 and practical experience from China. J Med Virol. 2020;92(7):755-769.

45. Pan F, Ye T, Sun P, et al. Time Course of Lung Changes at Chest CT during Recovery from Coronavirus Disease 2019 (COVID-19). Radiology. 2020;295(3):715-721.

46. De la Vega FM, Lazaruk KD, Rhodes MD, Wenz MH. Assessment of two flexible and compatible SNP genotyping platforms: TaqMan SNP Genotyping Assays and the SNPlex Genotyping System. Mutat Res. 2005;573(1-2):111-135.

47. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.

48. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-560.

49. Carrington M, Nelson GW, Martin MP, et al. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science. 1999;283(5408):1748-1752.

50. Thursz MR, Thomas HC, Greenwood BM, Hill AV. Heterozygote advantage for HLA class-II type in hepatitis B virus infection. Nat Genet. 1997;17(1):11-12.

51. Hraber P, Kuiken C, Yusim K. Evidence for human leukocyte antigen heterozygote advantage against hepatitis C virus infection. Hepatology. 2007;46(6):1713-1721.

52. Ricci C, Kakukram KR, Marzocchi C, et al. Thr92Ala polymorphism in the type 2 deiodinase gene: an evolutionary perspective. J Endocrinol Invest. 2020;43(12):1749-1757.

53. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383(2):120-128.

54. Ackermann M, Mentzer SJ, Kolb M, Jonigk D. Inflammation and intussusceptive angiogenesis in COVID-19: everything in and out of flow. Eur Respir J. 2020;56(5):2003147.

55. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):459-468.
56. Nair TS, Kakaraparthi BN, Yang L, et al. Slc44a2 deletion alters tetraspanin and N-cadherin expression: Reduced adhesion and enhanced proliferation in cultured mesenchymal lung cells. Tissue Cell. 2021;73:101599.

57. Bennett JA, Mastrangelo MA, Ture SK, et al. The choline transporter Slc44a2 controls platelet activation and thrombosis by regulating mitochondrial function. Nature Communications. 2020;11(1):1-9.

58. Bouhaddou M, Memon D, Meyer B, et al. The Global Phosphorylation Landscape of SARS-CoV-2 Infection. Cell. 2020;182(3):685-712.e19.

59. Dolskiy AA, Bodnev SA, Nazarenko AA, et al. Deletion of BST2 Cytoplasmic and Transmembrane N-Terminal Domains Results in SARS-CoV, SARS-CoV-2, and Influenza Virus Production Suppression in a Vero Cell Line. Frontiers in Molecular Biosciences. 2020;7:616798.

60. Xiong Y, Liu Y, Cao L, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerging Microbes and Infections. 2020;9(1):761-770.

61. Liao M, Liu Y, Yuan J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nature Medicine. 2020;26(6):842-844.

62. Coperchini F, Chiovato L, Ricci G, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: Further advances in our understanding the role of specific chemokines involved. Cytokine and Growth Factor Reviews. 2021;58:82-91.

63. Kim HJ, Jeong JS, Kim SR, Park SY, Chae HJ, LeeYC. Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF-κB/HIF-1α signaling pathway. Scientific Reports 2013 3:1. 2013;3(1):1-10.

64. Zeng M, Sang W, Chen S, et al. 4-PBA inhibits LPS-induced inflammation through regulating ER stress and autophagy in acute lung injury models. Toxicology letters. 2017;271:26-37.

65. McAninch EA, Jo S, Preite NZ, et al. Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated clinical syndromes. Journal of Clinical Endocrinology and Metabolism. 2015;100(3):920-933.
Legends

**Figure 1.** Flowchart of the study.

**Figure 2.** DIO2 Thr92Ala polymorphism and mortality (Cochran-Armitage test and Chi-square test); CI, confidence interval; OR, odds ratio; X², Chi-Square.

**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 metaanalysis (PRISMA 2020).

**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.

**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.
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)        | 63 (51-75)       | 60 (47-74)        | 63 (47-77)       | 0.5 | 1.0 | 0.993 – 1.07 | 0.17 |
| BMI (kg/m²)                       | 29.8 (26-34)   | 29.8 (27-33)     | 29.8 (25-34)      | 29.4 (26-34)     | 0.9 | 0.9 | 0.938 – 0.95 | 0.85 |
| Age > 60 year. n (%)              | 116 (52.7)     | 43 (65.6)        | 60 (50.4)         | 13 (56.5)        | 0.5 | 1.4 | 0.725 – 2.09 | 0.29 |
| Gender male. n (%)                | 135 (61.3)     | 41 (75.5)        | 79 (66.4)         | 15 (65.2)        | 0.1 | 0.6 | 0.319 – 0.22 | 0.15 |
| Length of hospital stay (d). median (IQR) | 6 (4-10) | 7 (4-11) | 6 (4-10) | 6 (4-12) | 0.6 | 1.0 | 1.052 – <0.0 | 0.001 |
| Hypertension. n (%)               | 144 (65.4)     | 58 (74.3)        | 72 (60.5)         | 14 (60.9)        | 0.0 | 1.0 | 0.565 – 0.67 | 0.40 |
| Diabetes mellitus. n (%)          | 93 (42.3)      | 33 (42.3)        | 51 (42.8)         | 9 (39.1)         | 0.8 | 1.3 | 0.661 – 1.0 | 0.40 |
| Cardiopathy. n (%)                | 28 (12.7)      | 12 (15.4)        | 15 (12.6)         | 1 (4.3)          | 0.6 | 0.3 | 0.052 – 0.14 | 0.14 |
| Chronic pneumopathy. n (%)        | 10 (4.5)       | 5 (6.4)          | 4 (3.3)           | 1 (4.3)          | 0.3 | 0.5 | 0.027 – 0.54 | 0.54 |
| Neoplasia. n (%)                  | 2 (0.9)        | 0 (0)            | 1 (0.8)           | 1 (4.3)          | 0.6 | 4.8 | 0.190 – 0.26 | 0.10 |
| Obesity. N (%) *                  | 103 (49)       | 37 (49)          | 55 (49.1)         | 11 (47.8)        | 0.9 | 0.3 | 0.169 – 0.02 | 0.05 |
| Complications                     |               |                 |                 |                 |     |     |                 |     |
| NTIS. N (%)                       | 14 (6.4)       | 4 (5.1)          | 7 (5.9)           | 3 (13)           | 0.0 | 4.1 | 1.268 – 0.01 | 0.05 |
| Cardiovascular shock. n (%)       | 28 (12.7)      | 12 (15.4)        | 13 (10.9)         | 3 (13)           | 0.3 | 54. | 19.3 – <0.0 | 0.001 |
| Endotracheal intubation. n (%)    | 29 (13.2)      | 14 (17.9)        | 12 (10)           | 3 (13)           | 0.1 | 12  | 39 – <0.0 | 0.001 |
| ICU admission. n (%)              | 55 (25)        | 24 (30.7)        | 26 (21.8)         | 5 (21.7)         | 0.3 | 36  | 14.8 – <0.0 | 0.001 |
| Scores systems                    |               |                 |                 |                 |     |     |                 |     |
| NEWS2 Score. median (IQR)         | 6 (5-7)        | 5 (4-6)          | 6 (5-7)           | 6 (5-7)          | 0.3 | 1.1 | 0.960 – 0.13 | 0.4 |
| q-SOFA Score. median (IQR)        | 1 (1-1)        | 1 (1-1)          | 1 (0-1)           | 1 (1-1)          | 0.4 | 1.8 | 0.892 – 0.10 | 0.10 |
| CT COVID Score. median (IQR)      | 20 (15-20)     | 20 (15-20)       | 20 (15-20)        | 20 (15-20)       | 0.7 | 1.0 | 0.972 – 0.27 | 0.05 |

Thr92Ala-DIO2 polymorphism and mortality (n=220).
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. BMI, body mass index; CT, computed tomography; ICU, intensive care unit; IQR, interquartile range; NEWS2, National Early Warning Score 2; NTIS, Non-Thyroid Illness Syndrome; qSOFA, quick Sepsis Related Organ Failure Assessment. * (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/T hr) (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)                   | 0.79 (0.9 – 8)                          |
|                           | 1.55 (0.9 – 2.9)                 | 1.19 (0.9 – 9)                          |
|                           | 1.62 (1.0 – 3.8)                 | 1.3 (0.6 – 5)                           |
| fT4 (0.89 - 1.72 ng/dL)   | 1.33 (1.1 – 1.6)                 | 0.49 (0.7 – 9)                          |
|                           | 1.35 (1.0 – 1.7)                 | 1.98 (0.7 – 9)                          |
|                           | 1.28 (0.8 – 1.5)                 |                                         |
| fT3 (2.0 - 4.2 pg/mL)     | 2.96 (2.6 – 3.4)                 | 0.27 (0.7 – 0)                          |
|                           | 2.92 (2.6 – 3.5)                 | 0.73 (0.3 – 0)                          |
|                           | 3.14 (2.1 – 3.2)                 | 0.2 (0.1 – 0)                           |
| rT3 (0.1 - 0.35 ng/mL)    | 0.48 (0.3 – 0.6)                 | 0.97 (0.1 – 0)                          |
|                           | 0.38 (0.2 – 0.6)                 | 1.00 (0.1 – 0)                          |
|                           | 0.52 (0.3 – 0.6)                 | 0.9 (0.1 – 0)                           |
| Thyreoglobulin (1.59-59.9 ng/mL) | 15.1 (6-26)  | 0.97 (0.1 – 0)                          |
|                           | 15.6 (6-28.7)                    | 0.97 (0.1 – 0)                          |
|                           | 14.5 (6-23)                      | 1.00 (0.1 – 0)                          |
|                           | 15.8 (6-45)                      | 0.9 (0.1 – 0)                           |
| FT3/FT3                   | 6.60 (4.6 – 9.9)                 | 0.96 (0.1 – 0)                          |
|                           | 7 (4.7 – 10.6)                   | 0.96 (0.1 – 0)                          |
|                           | 6.61 (4.7 – 9.8)                 | 1.00 (0.1 – 0)                          |
|                           | 4.83 (3.7 – 6.7)                 | 0.9 (0.1 – 0)                           |
| FT3*RT3                   | 1.28 (0.8-2.1)                   | 0.24 (0.1 – 0)                          |
|                           | 1.1 (0.7-1.9)                    | 0.71 (0.1 – 0)                          |
|                           | 1.46 (0.8-2.3)                   | 0.71 (0.1 – 0)                          |
|                           | 1.24 (0.7-1.9)                   | 0.71 (0.1 – 0)                          |
| FT4/FT3                   | 0.43 (0.3 – 0.5)                 | 0.99 (0.1 – 0)                          |
|                           | 0.45 (0.3 – 0.5)                 | 0.99 (0.1 – 0)                          |
|                           | 0.43 (0.3 – 0.6)                 | 0.99 (0.1 – 0)                          |
|                           | 0.48 (0.3 – 0.6)                 | 0.99 (0.1 – 0)                          |
| IL-6 (< 3.4 pg/mL)        | 49.8 (21-87)                     | 1.73 (0.7 – 0)                          |
|                           | 43 (23-83)                       | 1.73 (0.7 – 0)                          |
|                           | 55.5 (23-96)                     | 1.73 (0.7 – 0)                          |
|                           | 32.8 (19-84)                     | 1.73 (0.7 – 0)                          |
| D-dimer (<500 ng/mL)      | 759 (487-1628)                   | 1.00 (0.1 – 0)                          |
|                           | 924 (546-1579)                   | 1.00 (0.1 – 0)                          |
|                           | 696 (458-1496)                   | 1.00 (0.1 – 0)                          |
|                           | 706 (488-3629)                   | 1.00 (0.1 – 0)                          |
| LDH (207 - 414 U/L)       | 742 (538-522)                    | 1.00 (0.1 – 0)                          |
|                           | 723 (564-488)                    | 1.00 (0.1 – 0)                          |
|                           | 712 (488-5)                      | 1.00 (0.1 – 0)                          |
|                           | 718 (488-5)                      | 1.00 (0.1 – 0)                          |
Kruskal-Wallis test and Univariate logistic regression (mortality) were performed for all variables. 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.
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) |
|--------------------------|----------------------|----------------------|---------------------|-----------------------------------------------------|
|                          | O R* CI 95% P        | O R* CI 95% P        | O R* CI 95% P       | O R* CI 95% P                                       |
| Mortality                |                      |                      |                     |                                                     |
|                          | 0.18 - 0.26 1.75     | 0.23 0.6 1.06       | 0.36 0.9 3.57       | 0.52 0.26 - 1.04 0.07                                |
| Male gender              |                      |                      |                     |                                                     |
|                          | 0.18 - 0.26 1.76     | 0.24 0.9 1.02       | 0.34 0.9 3.43       | 0.54 0.26 - 1.08 0.08                                |
| Age > 60 year            |                      |                      |                     |                                                     |
|                          | 0.18 - 0.27 1.78     | 0.23 0.7 1.07       | 0.36 0.9 3.59       | 0.53 0.26 - 1.06 0.07                                |
| Diabetes                 |                      |                      |                     |                                                     |
|                          | 0.16 - 0.20 1.62     | 0.21 0.9 1.05       | 0.36 0.9 3.53       | 0.49 0.23 - 0.98 0.04                                |
| Hypertension             |                      |                      |                     |                                                     |
|                          | 0.18 - 0.26 1.75     | 0.23 0.7 1.07       | 0.36 0.9 3.55       | 0.53 0.26 - 1.06 0.08                                |
| Hospital stay            |                      |                      |                     |                                                     |
|                          | 0.15 - 0.21 1.65     | 0.21 0.7 1.07       | 0.32 0.9 3.39       | 0.51 0.24 - 1.05 0.07                                |
| Model 1                  |                      |                      |                     |                                                     |
|                          | 0.14 - 0.17 1.54     | 0.19 0.9 0.99       | 0.32 0.9 3.51       | 0.46 0.21 - 0.98 0.04                                |
| TSH                      |                      |                      |                     |                                                     |
|                          | 0.15 - 0.24 1.72     | 0.23 0.6 0.04       | 0.35 0.9 3.51       | 0.52 0.26 - 1.05 0.07                                |
| Free T3                  |                      |                      |                     |                                                     |
|                          | 0.17 - 0.26 1.74     | 0.25 0.9 0.37       | 0.62 0.3 - 1.28     | 0.20                                                 |
|       | Free T4  | Reverse T3 | Model 2  | IL6    | CRP    | D-DIMER | Neutrophil | LDH    | Model 3  | Model 4  | Model 5  |
|-------|----------|------------|----------|--------|--------|----------|------------|--------|----------|----------|----------|
|       | 0.65     | 0.16       | 0.48     | 0.57   | 0.52   | 0.56     | 0.47       | 0.46   | 0.52     | 0.55     | 0.56     |
|       | 0.51     | 0.19       | 0.19     | 0.16   | 0.14   | 0.26     | 0.20       | 0.18   | 0.34     | 0.15     | 0.32     |
|       | 0.00     | 0.10       | 0.00     | 0.00   | 0.00   | 0.00     | 0.00       | 0.00   | 0.00     | 0.00     | 0.00     |
|       | 0.00     | 0.00       | 0.00     | 0.00   | 0.00   | 0.00     | 0.00       | 0.00   | 0.00     | 0.00     | 0.00     |
|       | 0.00     | 0.00       | 0.00     | 0.00   | 0.00   | 0.00     | 0.00       | 0.00   | 0.00     | 0.00     | 0.00     |
Multivariable regression analyses – Model 1 - adjusted for gender male, age > 60 anos, diabetes e arterial hypertension and and length of hospital stay; Model 2 - adjusted for TSH, fT3, fT4 and rT3; Model 3 - adjusted for IL6, Neutrophil, LDH, CRP and D-dimer; Model 4 - adjusted for Model 1 and 3; Model 5 - adjusted for all of the above variables.
Table 4. Fixed and Random effects meta-analysis of the haplotype association between the occurrence of a series of diseases and the Thr92Ala-DIO2 polymorphism according to three inheritance models: dominant, overdominant and recessive.

| Author, year   | N   | Major endpoint                  | Dominant model (Thr/Thr vs Thr/Ala + Ala/Ala) | Overdominant model (Thr/Ala vs Thr/Thr + Ala/Ala) | Recessive model (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                          | 0.4 1.41 (0.62 - 3.19)                         | 0.6 0.84 (0.40 - 1.75)                          | 0.7 0.86 (0.37 - 1.99)                       |
| 2. Kang, 2020  | 602 | Osteoporosis                    | 0.4 0.82 (0.50 - 1.34)                         | 0.7 0.90 (0.58 - 1.41)                          | 0.1 1.48 (0.89 - 2.48)                       |
| 3. Galecka, 2015 | 331 | Recurrent depressive disorder   | 0.5 1.16 (0.75 - 1.79)                         | 0.6 1.13 (0.73 - 1.74)                          | 0.01 0.07 (0.01 - 0.56)                      |
| 4. Lee, 2015   | 809 | Alcohol dependence             | 0.7 0.94 (0.70 - 1.27)                         | 1.0 0.99 (0.74 - 1.32)                          | 0.6 1.12 (0.76 - 1.63)                       |
| 5. Luo, 2015   | 260 | Mild cognitive impairment      | 0.8 0.92 (0.55 - 1.55)                         | 0.9 0.97 (0.59 - 1.58)                          | 0.6 1.16 (0.65 - 2.06)                       |
| 6. Huang, 2013 | 469 | Kashin-Beck disease             | 0.8 1.06 (0.71 - 1.60)                         | 0.9 0.98 (0.64 - 1.50)                          | 0.6 0.70 (0.22 - 2.23)                       |
| 7. Nair, 2012  | 300 | Diabetes (Pima Indians)         | 0.2 0.43 (0.13 - 1.43)                         | 0.05 0.60 (0.36 - 1.01)                         | 0.01 1.86 (1.13 - 3.04)                      |
| 8. Xiong, 2010 | 370 | Kashin-Beck disease             | 0.9 0.96 (0.81 - 1.51)                         | 0.7 0.91 (0.61 - 1.38)                          | 0.5 1.21 (0.73 - 2.02)                       |
| 9. Dora, 2010  | 157 | Diabetes                        | 0.5 0.93 (0.75 - 1.15)                         | 0.3 0.90 (0.73 - 1.11)                          | 0.02 1.43 (1.05 - 1.96)                      |
| 10. He, 2009   | 558 | Bipolar disorder               | 0.00 0.61 (0.42 - 0.88)                         | 0.4 0.87 (0.62 - 1.21)                          | 0.00 2.27 (1.50 - 3.44)                      |
| 11. Meulenberg, 2008 | 311 | Osteoarthritis                 | 0.9 1.01 (0.86 - 1.18)                         | 0.00 0.79 (0.67 - 0.91)                         | <0.0 1.64 (1.32 - 2.04)                      |
| 12. Van der Deur, 2008 | 229 | Hypertension                    | 0.7 1.04 (0.88 - 1.23)                         | 0.6 1.04 (0.88 - 1.23)                          | 0.2 0.84 (0.66 - 1.07)                       |
| 13. Maia, 2007 | 163 | Diabetes                        | 0.7 1.06 (0.77 - 1.47)                         | 0.4 0.86 (0.63 - 1.19)                          | 0.4 1.22 (0.78 - 1.92)                       |
| 14. Gumieniak, 2007 | 372 | Hypertension                    | 0.00 0.48 (0.28 - 0.82)                         | 0.00 2.30 (1.31 - 4.03)                         | 0.7 0.85 (0.41 - 1.76)                       |
| 15. Grarup, 2006 | 617 | Diabetes                        | 0.3 1.06 (0.94 - 1.20)                         | 0.1 0.91 (0.81 - 1.03)                          | 0.4 1.08 (0.90 - 1.28)                       |
| 16. Grarup, 2006 | 584 | Obesity                         | 0.1 1.11 (0.97 - 1.28)                         | 0.8 0.98 (0.86 - 1.13)                          | 0.1 0.82 (0.66 - 1.02)                       |
| 17. Mentuccia, 2008 | 126 | Diabetes                        | 0.5 0.91 (0.86 - 1.24)                         | 0.7 0.95 (0.65 - 1.30)                          | 0.1 1.52 (0.93 - 2.49)                       |
| 18. Guo, 2004  | 538 | Mental retardation              | 0.7 1.07 (0.75 - 1.52)                         | 0.6 0.69 (0.48 - 0.99)                          | 0.9 1.03 (0.65 - 1.65)                       |
| Metanalysis total (Fixed) | 275 |                                | 0.7 1.01 (0.95 - 1.07)                         | 0.01 0.93 (0.88 - 0.96)                         | 0.00 1.15 (1.06 - 1.24)                      |
| Metanalysis total (Random) | 275 |                                | 0.7 0.99 (0.92 - 1.07)                         | 0.05 0.93 (0.87 - 1.00)                         | 0.04 1.19 (1.01 - 1.41)                      |

Heterogeneity analysis

| Tau² | Chi² | P | I² |
|------|------|---|----|
| 0.01 | 22.43| 0.17| 24%|
| 0.00 | 21.64| 0.20| 21%|
| 0.07 | 53.73|<0.0001| 68%|

Number of studies with p ≤ 0.05

3 studies 3 studies 5 studies
| Advantage (n) | Disadvantage (n) |
|--------------|------------------|
| 2            | 1/4              |

CI, confidence interval; OR, odds ratio;
Table 5. Fixed and Random effects meta-analysis of the haplotypic association between disease outcome and the Thr92Ala-DIO2 polymorphism according to three inheritance models: dominant, overdominant and recessive.

| Author, year       | N  | Major endpoint                      | Dominant model (Thr/Thr vs Thr/Ala + Ala/Ala) | Overdominant model (Thr/Ala vs Thr/Thr + Ala/Ala) | Recessive model (Ala/Ala vs Thr/Ala + Thr/Thr) |
|--------------------|----|-------------------------------------|-----------------------------------------------|-------------------------------------------------|-----------------------------------------------|
|                    |    |                                     | P value | OR (95% CI)                          | P value | OR (95% CI)                          | P value | OR (95% CI)                          |
| 1. Beltrao, 2021   | 22 | COVID-19 death                     | 0.1     | 1.83 (0.90 - 3.71)                  | 0.05    | 0.49 (0.24 - 1.00)                  | 0.6     | 1.38 (0.48 - 3.98)                  |
| 2. Taroza, 2020    | 16 | Depression after AIS               | 0.3     | 2.20 (0.47 - 10.2)                  | 0.4     | 0.72 (0.36 - 1.43)                  | 0.6     | 1.21 (0.61 - 2.40)                  |
| 3. Kazukauskiene, 2020 | 28 | Cardiac-related death in ICU       | 0.1     | 2.52 (0.77 - 8.23)                  | 0.3     | 0.54 (0.16 - 1.75)                  | 0.6     | 0.42 (0.02 - 7.29)                  |
| 4. Taroza, 2019    | 24 | AIS severity                       | 0.4     | 1.53 (0.52 - 4.49)                  | 0.04    | 0.58 (0.35 - 0.98)                  | 0.1     | 1.54 (0.92 - 2.57)                  |
| 5. Grineva, 2009   | 14 | LVH with Graves’s disease          | 0.00    | 3.06 (1.36 - 6.90)                  | 0.02    | 0.36 (0.15 - 0.87)                  | 0.4     | 0.49 (0.10 - 2.34)                  |
| Metanalysis total (Fixed) | 62 |                                   | 0.00    | 2.18 (1.43 - 3.3)                   | 0.00    | 0.54 (0.40 - 0.87)                  | 0.20    | 1.27 (0.88 - 1.83)                  |
| Metanalysis total (Random) | 62 |                                   | 0.00    | 2.17 (1.41 - 3.3)                   | 0.00    | 0.55 (0.40 - 0.87)                  | 0.17    | 1.30 (0.90 - 1.88)                  |
| Heterogeneity analysis |       |               | Tau² = 0.00; Chi² = 1.37, P = 0.85; I² = 0% | Tau² = 0.00; Chi² = 1.57, P = 0.81; I² = 0% | Tau² = 0.00; Chi² = 2.57, P = 0.63; I² = 0% |
| Number of studies with p ≤ 0.05 |       |               | 1 studies                          | 3 studies                         | 0 studies                          |
| Advantage (n)/Disadvantage (n) |   |               | 0/1                                | 3/0                                | 0/0                                |

AIS, Acute ischemic stroke; CI, confidence interval; ICU, intensive care unit; LVH, left ventricular hypertrophy; OR, odds ratio;
Figure 1

Assessed for eligibility (n=274)

At triage

Patients included in the analysis (n=245)

Patients with genotyping data (n=220)

Excluded patients: 29
Meeting exclusion criteria (n=26)
- Use of drugs that interfere with thyroid metabolism (n=13)
- Use of iodinated contrast in the last six months (n=5)
- Withdrawal due to overt and/or subclinical hypothyroidism (n=6)
Withdrawal due to transfer of hospital (n=3)

Patients without genotyping data available (n=25)

Ala/Thr (n=119)  Thr/Thr (n=78)  Ala/Ala (n=23)

Non-survivors
(n=15)  Non-survivors
(n=18)  Non-survivors
(n=5)

Survivors
(n=104)  Survivors
(n=60)  Survivors
(n=18)
Figure 2

| Inheritance models | Genotype | Non survivor (n) | Mortality (%) | Chi-square or Cochran-Armitage test | OR (95% CI)* | P value |
|--------------------|----------|-----------------|---------------|-------------------------------------|--------------|---------|
| Overall            | TT + TC + CC | 39/220          | 17.3%         | -                                   | -            | -       |
| Codominant         | TT       | 18/78           | 23.1%         | $\chi^2 = 3.75, P = 0.05$           | 1            | 0.06    |
|                    | TC       | 15/119          | 12.6%         |                                      | 0.48         | 0.91    |
|                    | CC       | 5/23            | 21.7%         |                                      | 1.06         | -       |
| Dominant           | TT + CC  | 18/78           | 23.1%         | $\chi^2 = 2.84, P = 0.09$           | 1.80         | 0.1     |
|                    | CC + TC  | 20/142          | 14.1%         |                                      | 0.55 (0.27 – 1.13) | -     |
| Recessive          | TC + TT  | 33/197          | 16.6%         | $\chi^2 = 0.35, P = 0.54$           | 0.72         | 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         | 0.05    |
|                    | TC       | 15/119          | 12.6%         |                                      | 0.49 (0.23 – 1.00) | -     |

Mortality (%)
Figure 3

A. Kaplan-Meier plot showing the probability of survival over time for different genotypes. The chi-squared test statistic is $\chi^2 = 3.02$ with a p-value of 0.082.

B. Kaplan-Meier plot showing the probability of survival over time for different genotypes. The chi-squared test statistic is $\chi^2 = 3.91$ with a p-value of 0.0481. The hazard ratio (HR) is 0.52 (95% CI: 0.28-0.99).

N at risk:
- Thr/Thr: 119, 115, 106, 105, 104
- Thr/Ala: 78, 71, 66, 61, 60
- Ala/Ala: 23, 21, 19, 18

N at risk:
- Thr/Thr + Ala/Ala: 119, 115, 106, 105, 104
- Thr/Thr: 101, 92, 85, 79, 78
- Ala/Ala: 18
Figure 4

Identification of studies via database and registers

Records identified from:
- Databases (n=97)
  - Pubmed (n=88)
  - Cochrane (n=8)
  - Scielo (n=1)

Records removed before screening:
- Duplicate records removed (n=5)

Records screened (n=92)
- Review articles (n=12)
- Thyroid dysfunction (n=29)

Records screened (n=41)
- Reports not retrieved (n=0)

Records assessed for eligibility (n=51)
- Reports excluded (n=30)
  - Without control group (n=20)
  - Small sample size (n=4)
  - Wrong polymorphism (n=8)

Studies included in review and metaanalysis (n=21)
### Figure 5

| Study or Subgroup                                | Diseases Events | Controls Events | Total Events | M-H, Random 95% CI | M-H, 95% CI |
|--------------------------------------------------|-----------------|-----------------|--------------|---------------------|-------------|
| Maroones 2021 (Autistic spectrum disorder)       | 39              | 90              | 20           | 0.9%                | 0.84 [0.40, 1.75] |
| Kang 2020 (Osteoporosis)                         | 38              | 83              | 1383         | 2.4%                | 0.90 [0.58, 1.41] |
| Galatka 2015 (Depressive disorder)               | 95              | 179             | 76           | 2.5%                | 1.13 [0.73, 1.74] |
| Lee 2015 (Alcohol dependence)                    | 245             | 521             | 136          | 5.1%                | 0.99 [0.74, 1.32] |
| Luo 2015 (Mild cognitive impairment)             | 57              | 129             | 59           | 131 2.0%            | 0.97 [0.59, 1.58] |
| Huang 2013 (Kashin-Beck disease)                 | 56              | 256             | 56           | 2.6%                | 0.96 [0.66, 1.40] |
| Nair 2012 (Diabetes - Pima Indians)              | 34              | 150             | 49           | 150 1.8%            | 0.80 [0.49, 1.31] |
| Xiong 2010 (Kashin-Beck disease)                 | 78              | 161             | 106          | 209 2.7%            | 0.91 [0.61, 1.38] |
| Dora 2010 (Diabetes)                             | 503             | 1057            | 259          | 518 8.3%            | 0.90 [0.73, 1.11] |
| He 2009 ( Bipolar disorder)                      | 130             | 274             | 145          | 284 4.0%            | 0.87 [0.62, 1.22] |
| Meulenbelt 2008 (Osteoarthritis)                 | 412             | 979             | 1026         | 2155 12.6%          | 0.79 [0.67, 0.92] |
| van der Deur 2008 ( Hypertension)                | 492             | 1020            | 601          | 1274 11.5%          | 1.04 [0.88, 1.23] |
| Maia 2007 (Diabetes)                             | 76              | 169             | 712          | 1462 4.3%           | 0.80 [0.63, 1.19] |
| Gunerken 2007 ( Hypertension)                    | 154             | 304             | 21           | 68 1.5%             | 2.30 [1.31, 4.03] |
| Granup 2006 ( Diabetes)                          | 618             | 1405            | 2200         | 4770 16.1%          | 0.91 [0.81, 1.03] |
| Granup 2006 (Obesity)                            | 438             | 987             | 2230         | 4876 14.0%          | 0.98 [0.86, 1.13] |
| Mangucca 2005 (Diabetes)                         | 73              | 179             | 459          | 1089 4.2%           | 0.98 [0.86, 1.13] |
| Guo 2004 (Mental retardation)                    | 87              | 208             | 145          | 330 3.6%            | 0.92 [0.65, 1.30] |
| Total (95% CI)                                   | 8111            | 19392           | 100.0%       | Random 0.03 [0.87, 1.00] |

**Heterogeneity:** Tau² = 0.00, Chi² = 21.64, df = 17 (P = 0.020), I² = 21%

**Random Test for overall effect:** Z = 1.93 (P = 0.05)

**Fixed Test for overall effect:** Z = 2.53 (P = 0.01)
Figure 6

| Study or Subgroup | Diseases | Controls | Odds Ratio |
|-------------------|----------|----------|------------|
|                   | Events   | Total    |            | M-H, Fixed, 95% CI |
| Beltrao 2021 (COVID-19 death) | 15 38 | 104 182 | 0.49 [0.24, 1.00] |
| Tanua 2020 (Depression after IS) | 18 44 | 61 124 | 0.72 [0.36, 1.43] |
| Kazuksaikene 2020 (AMI death) | 4 14 | 115 269 | 0.54 [0.16, 1.79] |
| Tanua 2019 (Acute ischemic stroke severity) | 57 153 | 48 95 | 0.58 [0.35, 0.98] |
| Grineva 2009 (LVH with Gravel’s disease) | 8 40 | 42 103 | 0.36 [0.15, 0.87] |
| Total (95% CI) | 289 773 | 370 | 100.0% | Fixed 0.54 [0.40, 0.75] |
| Total events | 102 | 370 | Random 0.55 [0.40, 0.75] |

Heterogeneity: Chi² = 1.57, df = 4 (P = 0.81), I² = 0%
Favours [non-disease] Favours [disease]
Random Test for overall effect: Z = 3.70 (P = 0.0002)