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RESEARCH

Impact of SNP Variants in *PON-1* or *UGT1A1* on Iron Chelation Therapy Outcomes and Zinc Status in Thalassemia Major Patients

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Abstract

Factors affecting iron chelation therapy outcomes are complex and should be identified to tailor interventions to the needs of individuals with beta-thalassemia major (TM). The purpose of the study was to determine the effects of *PON-1* or *UGT1A1* single-nucleotide polymorphisms on therapeutic outcomes via deferasirox (DFX) and the antioxidant status. *PON-1* (rs662) or *UGT1A1* (rs887829) polymorphisms, iron chelation therapy outcomes (cardiac iron T2*, serum ferritin (SF)), and antioxidant-related nutritional indices (PON-1 activity, zinc, 25-hydroxyvitamin D) were determined in 44 Taiwanese TM patients receiving chronic blood transfusion and DFX therapy. Patients' cardiac iron T2* values were negatively correlated with SF levels ($r = -0.38$, $p < 0.01$). *PON-1* AA/AG carriers had significantly greater PON-1 activity, whereas *PON-1* GG carriers were prescribed significantly higher DFX doses. *UGT1A1* CT and TT carriers had marginally significantly greater SF levels. Only four patients had normal levels of 25-hydroxyvitamin D (25(OH)D > 30 ng/mL). PON-1 activity in those with SF > 2500 (6.4 ± 1.9 units/mL) was significantly lower than that (7.7 ± 1.7 units/mL; $p < 0.03$) in patients with SF ≤ 2500. Although not statistically significant, variants in *PON-1* or *UGT1A1* were associated with increased odds ratios (2.44 and 2.899, respectively) for lower cardiac iron T2* values < 30 ms. Taiwanese TM patients with moderate iron overload status had significantly lower PON-1 activity and vitamin 25(OH)D levels, particularly those with T2* < 30 ms. Patients with *PON-1* GG and *UGT1A1* TT carriers may have an increased risk of cardiac iron overload.

Keywords Beta-thalassemia major · Polymorphisms · Iron chelation outcomes · PON-1 activity · Zinc · Vitamin D

Introduction

Beta-thalassemia major (TM) patients received blood transfusion therapy at routine intervals and may develop chronic iron overload syndrome; however, the outcome of iron therapy is still not satisfactory under high-dose deferasirox (DFX) treatment. A possible explanation is that 36% of TM patients treated with DFX are intolerant or have a poor response to DFX treatment [1]. Another potential factor is pharmacokinetics [2], and drug metabolic variations in single-nucleotide polymorphisms (SNPs) have been examined in the USA. More recently, serial reports by Allegra et al. [3–7] demonstrated that genetic variants (including in genes encoding liver enzymes such as CYPs, UGTs, or vitamin D pathway genes) contribute to iron chelation outcomes in Italian TM patients. Considering the outcomes of iron controls, a lower serum ferritin (SF) concentration was observed in patients who carried the *UGT1A1* rs887829 C > T TT genotype than in those who carried the CC/CT genotype. Overall, the impact of SNPs on iron chelation outcomes and the frequency of minor alleles in Taiwanese TM patients are unclear.

In living organisms, zinc is the second most abundant trace element after iron. To evaluate patient outcomes during iron chelation therapy, several blood parameters, including zinc and antioxidant enzymes such as PON-1, in addition to SF and heart T2*, have been suggested for use in clinical practice. The biological functions and pivotal roles of zinc include acting as a cofactor for many enzymes involved in bone absorption/metabolism, immunology, wound healing, and insulin biosynthesis [8, 9]. Both zinc [10, 11] and PON-1 activity [12, 13] have been investigated in TM patients. Increased zinc excretion was further supported

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by a recent prevalence or incidence data showing TM with hypozincemia (< 700 mcg/L) ranges from 26% (North America [14]) to 80% (Iran [15], Sri Lanka [16]). Antioxidant enzyme imbalance is reported to be a consequence of iron chelation medication, as the serum PON-1 activity in patients with TM is reportedly lower than that in healthy control individuals.

Additionally, PON-1 enzyme is considered to hydrolyze lipid peroxidation products and is therefore a biomarker of diseases involving oxidative stress [17]. PON-1 activity is closely associated with high-density [18] and low-density [19] lipoprotein levels, and reduced PON-1 activity has been linked to increased cardiovascular risk. Interestingly, PON-1 activity can be improved in individuals with the PON-1–Q192R homozygous QQ genotype (Q allele, genotype AA) following hypoxic training [20]. Carriers of the Q allele tend to exhibit a more antiatherogenic lipid and lipoprotein profile compared to R allele carriers, including lower homocysteine levels and enhanced antioxidant protection in cardiovascular cohorts. However, our previous study [21] found that Q allele carriers had significantly lower 5-methyltetrahydrofolate (5-MTHF) concentrations. This may be due to the presence of the MTHF reductase C allele, which is associated with reduced MTHF availability for the remethylation of homocysteine to methionine—highlighting that PON-1 activity may be influenced by additional biochemical pathways or cofactors. For example, S-adenosylmethionine (SAM), a key methyl donor, supports trans-sulfuration and glutathione synthesis—an essential antioxidant that helps preserve PON-1 activity. In states of zinc deficiency, downregulation of enzymes such as methionine synthase and betaine-homocysteine methyltransferase can occur, leading to elevated homocysteine levels and depletion of SAM. These disruptions in one-carbon metabolism may further impact PON-1 activity [9].

Zinc plays multiple essential roles in human metabolism. In addition to serving as a cofactor in the one-carbon cycle—thereby indirectly supporting PON-1 functions—zinc is also critically involved in the biological effects of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) [22]. The vitamin D receptor is similar to other steroid receptors and can specifically interact with zinc finger regions [23] and also influence hepcidin expression. In addition, glucuronidation is an essential chemical reaction that converts $1,25(OH)_2D_3$ into a biologically inactive form through the action of UGT1A enzymes. Among these, UGT1A1 not only contributes to vitamin D metabolism but is also thought to interact functionally with both $1,25(OH)_2D_3$ and bilirubin [24], each of which contributes to the regulation of iron metabolism. Previous studies have also demonstrated a correlation between iron overload and vitamin D deficiency in patients with thalassemia [3, 6]. These findings highlight a complex interplay among zinc status, vitamin D metabolism, PON-1 activity, and UGT1A1 function—factors that may collectively influence iron homeostasis and therapeutic outcomes in patients with thalassemia major.

Therefore, the aim of the current study was to investigate the impact of *PON-1* and *UGT1A1* single-nucleotide polymorphisms (SNPs) on the therapeutic outcomes of the iron chelator DFX with a focus on serum ferritin levels and cardiac iron status as indicated by T2* values. Additionally, differences in blood biochemical parameters—particularly antioxidant-related nutritional indices such as PON-1 activity, zinc, and vitamin D levels—were evaluated among Taiwanese TM patients with varying responses to iron chelation therapy.

Materials and Methods

Patient Information

The inclusion criteria for patients were a diagnosis of TM at an age older than 14 years and the requirement of routine blood infusion together with regular therapy comprising the iron chelator DFX. Patients who were pregnant were excluded. This clinical protocol was approved by the Research Ethics Committee (REC) of National Taiwan University Hospital (NTUH) and was conducted as set out in the protocol (approval ID 201401095RINC). Patients who provided informed consent were included in the current study; their therapy outcomes were retrospectively evaluated, and their blood samples were prospectively collected into different tubes for SNP identification. Related laboratory data and biochemistry tests, including efficacy, complications, PON-1 activity, and related nutrients, were used to determine patients' pharmacotherapy outcomes and antioxidant status.

Outcome Measurements

Pharmacotherapy outcomes included treatment efficacy, complications, and PON-1 activity and related nutrients. Therapeutic outcome measurements included SF, serum iron, TIBC, cardiac T2* (reference, 40–64 ms; < 20 ms indicates microvascular injury), and hormones such as testosterone, estrogen, FSH, and LH. Nutritional trace elements, such as serum calcium, zinc, iron, and vitamin D, were measured. Blood samples were centrifuged within 30 min to obtain the serum, which was either delivered to the clinical laboratory for determination tests or stored at − 80 °C for PON-1 activity determination via an EnzChek® Paraoxonase Assay Kit (Molecular Probes, Invitrogen, USA). The procedures used were modified from protocols obtained from the literature [25] and from the manufacturers. The calibration curve ranged between 0.1 μM (0.01 nmol) and 6 μM (0.6 nmol)

through fluorometry with SkanIt™ Software; the excitation/emission wavelengths were set to 360 nm/450 nm.

Determination of Polymorphisms

DNA was extracted with a QIAamp DNA Blood Mini Kit, and polymorphisms in *PON-1* were detected. Polymorphism variants were then determined via similar procedures [26] (TaqMan™ Genotyping Master Mix or TaqMan™ SNP Genotyping Assay) and further analyzed with Applied Biosystems Sequence Detection Systems (version 2.4.1), as described in Lu et al. [21]. The distribution of PON-1 rs662 polymorphisms among the study population was as follows: 23% were wild-type (GG), 39% were heterozygous (GA), and 39% were homozygous variant (AA). For the UGT1A1 rs887829 polymorphism, 73% of patients were wild-type (TT), 23% were heterozygous (TC), and 5% were homozygous variant (CC).

Statistical Analyses

(1) Descriptive statistics: Demographic and baseline clinical characteristics were summarized using means or medians, as appropriate, along with standard descriptive statistics. (2) Correlation analyses: Spearman's correlation tests were used to evaluate associations between serum ferritin (SF) and key clinical variables, including cardiac iron (T2*), iron chelator dose, total iron-binding capacity (TIBC), and antioxidant-related parameters. Additional correlations between SF levels and patients' biochemical markers were also assessed. (3) Based on previous findings [27], an SF threshold of 2500 ng/mL was used to define subgroups, as elevated SF levels have been linked to increased risk of severe cardiac iron overload and reduced survival. Patients were accordingly stratified into two groups ($\leq$2500 ng/mL vs. > 2500 ng/mL), and comparisons were made for PON-1 activity, zinc, and 25(OH)D.

Differences in iron indicators, PON-1 activity, and zinc levels were further analyzed across *PON-1* and *UGT1A1* genotype groups (wild-type, heterozygous, and homozygous). For specific comparisons, heterozygous individuals were grouped with either the wild-type or homozygous variant group, depending on the context of the analysis. (4) Genotype-based analysis: To assess the impact of single-nucleotide polymorphisms (SNPs) on iron overload status, median values of various iron chelation therapy outcomes were calculated and compared among different genotype groups.

Group differences were assessed using Student's *t*-test or one-way ANOVA for normally distributed data. When normality assumptions were not met, data were log-transformed before statistical testing. Non-parametric tests were then applied where appropriate, including the Mann–Whitney *U* test for two-group comparisons and the Kruskal–Wallis test for comparisons involving more than two groups. Multiple logistic regression analysis was performed to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of variables (serum ferritin, DFX total daily dose, Zn, 25(OH)D, PON-1 activity, PON-1 rs662 G allele, UGT1A1 rs887829 T allele) with cardiac iron control outcomes, using serum ferritin as the reference (OR = 1). All statistical analyses were conducted using SigmaPlot software (version 12; Systat Software Inc., San Jose, CA, USA). A p-value of < 0.05 was considered statistically significant.

Results

A total of 44 patients were included, and their demographic characteristics and laboratory data are summarized in Table 1. Further analysis based on different ranges of serum ferritin as iron overload status showed that approximately 30% had an SF concentration less than 1000 ng/mL (mean value, 657.2 ± 162.8 ng/mL), which is considered good management under iron chelation therapy and close-dose titration by routine clinical care. However, the other 70% of patients needed close follow-up treatment compliance or factor intervention during chelation therapy, especially those patients ($n = 13$) whose SF levels ranged from 1000 to 2500 ng/mL (mean value, 1468.5 ± 440.9 ng/mL) or > 2500 ng/mL (mean value, 6939.5 ± 5988.9 ng/mL). A total of 80% of patients ($n = 35$) were considered to have normal zinc levels (in Taiwan, 700 mcg/L is considered the lower margin), and the mean serum zinc concentration was 828.6 ± 106.6 mcg/L (min–max, 700–1204) in comparison to those considered to have hypozincemia (628.0 ± 41.9; min–max, 557–692 mcg/L).

Spearman's correlation tests of patient SF levels and the dose of iron chelators revealed a significant correlation between the levels and the daily total dose ($r = 0.48$, $p < 0.001$). Similar tests were also applied, and results showed that patients' SF levels were significantly negatively correlated with cardiac iron T2* values ($r = -0.38$, $p < 0.01$), TIBC ($r = -0.35$, $p < 0.05$). However, despite a negative trend, the SF concentration was not significantly correlated with the serum zinc, PON-1 activity, or vitamin D concentrations ($r = -0.10, -0.25$, and -0.13, respectively).

As shown in Fig. 1, the mean PON-1 activity of these patients (SF $\leq$ 2500) was 6.4 ± 1.9 units/mL, which was significantly lower than that (7.7 ± 1.7 units/mL) of patients whose SF level was > 2500 ($p < 0.05$). Subgroup analysis of patients stratified according to the *PON-1* rs662 polymorphism revealed that it had an impact on dose selection and outcome parameters, in addition to PON-1 activity. As shown in Fig. 2, PON-1 activity was significantly greater in patients who carried *PON-1* rs662 AA/AG (8.1 ± 1.7 Unit/mL) than in those who carried *PON-1* rs662 GG (6.2 ± 1.4

Table 1 Patient demographic characteristics ($n=44$)

Characteristics	Mean ± SD (min–max)
Sex (M:F), *n* (%)	21 (48):23 (52)
Age (year)	33.4 ± 6.9
Weight (kg)	54.5 ± 9.2
Serum ferritin (ng/mL), median (IQR)	1320.8 (805.9–3134.4)
Cardiac T2* values (ms), median (IQR)	44.0 (27.0–57.0)
DFX dose (ng/kg/day), median (IQR)	30.3 (23.9–35.3)
DFX total daily dose (mg/day), median (IQR)	1625.0 (1250.0–1843.8)
Monitoring of chelation therapy	
Cardiac T2* values (ms)	42.1 ± 19.9 (7.0–85.0)
Ferritin (ng/mL)	2845.2 ± 4171.6 (276.4–24146.2)
Iron (mcg/dL)	262.0 ± 67.8 (126.0–421.0)
TIBC (mcg/dL)	555.0 ± 243.5 (162.0–1051.0)
Transferrin saturation (%)	55 ± 21 (23–92)
PON-1 activity and its related nutrients	
Zn (mcg/L)	787.6 ± 126.5 (557.0–1204.0)
P (mg/dL)	4.1 ± 0.7 (2.9–6.2)
Total 25-OH vitamin D (ng/mL)	16.8 ± 6.9 (5.9–38.9)
PON-1 (Unit/mL)	7.3 ± 1.9 (3.7–12.0)
Sex hormones	
FSH (mIU/mL)	5.9 ± 5.4 (0.3–26.7)
LH (mIU/mL)	4.2 ± 6.8 (0.1–42.9)
Estradiol (E2) (pig/mL)	58.0 ± 56.3 (11.8–358.3)
Estriol (E3) (ng/mL)	0.093 ± 0.052 (0.020–0.243)
Testosterone (ng/dL)	178.8 ± 244.6 (8.9–784.8)

DFX deferasirox, *IQR* interquartile range

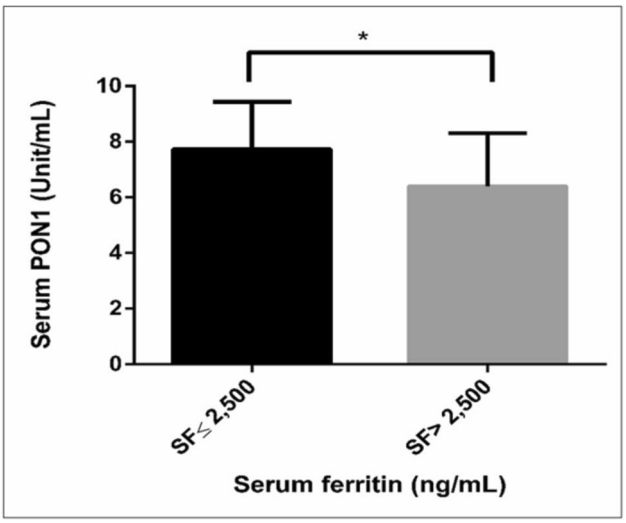

Fig. 1 Comparison of PON-1 activity in thalassemia major patients stratified by serum ferritin (SF) levels. Patients were divided into two subgroups based on SF levels: > 2500 ng/mL and ≤ 2500 ng/mL. PON-1 activity was significantly different between the groups. Data are presented as mean ± SD. *$p<0.05$ was considered statistically significant

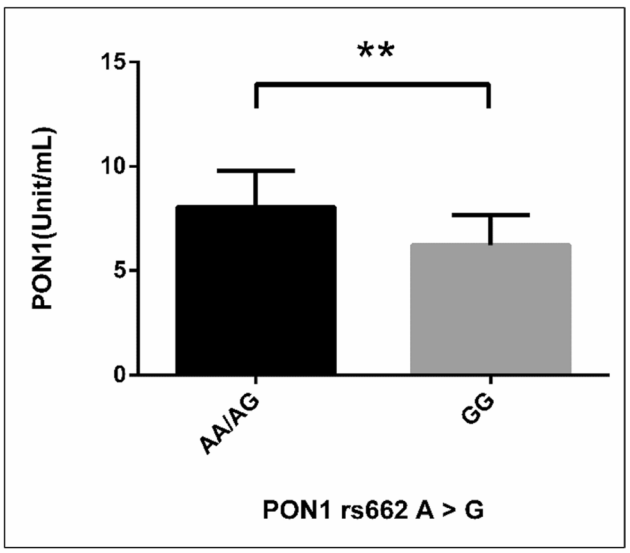

Fig. 2 Comparison of clinical and biochemical variables in thalassemia major (TM) patients stratified by PON-1 rs662 genetic polymorphism. **A** PON-1 enzymatic activity; **B** iron chelation parameters, including daily dose of deferasirox, total daily dose, transferrin saturation, and serum ferritin levels. Data are presented as medians. Statistically significant differences were observed between patients with the rs662 GG genotype and other TM patients. *$p<0.05$; **$p<0.01$ was considered statistically significant

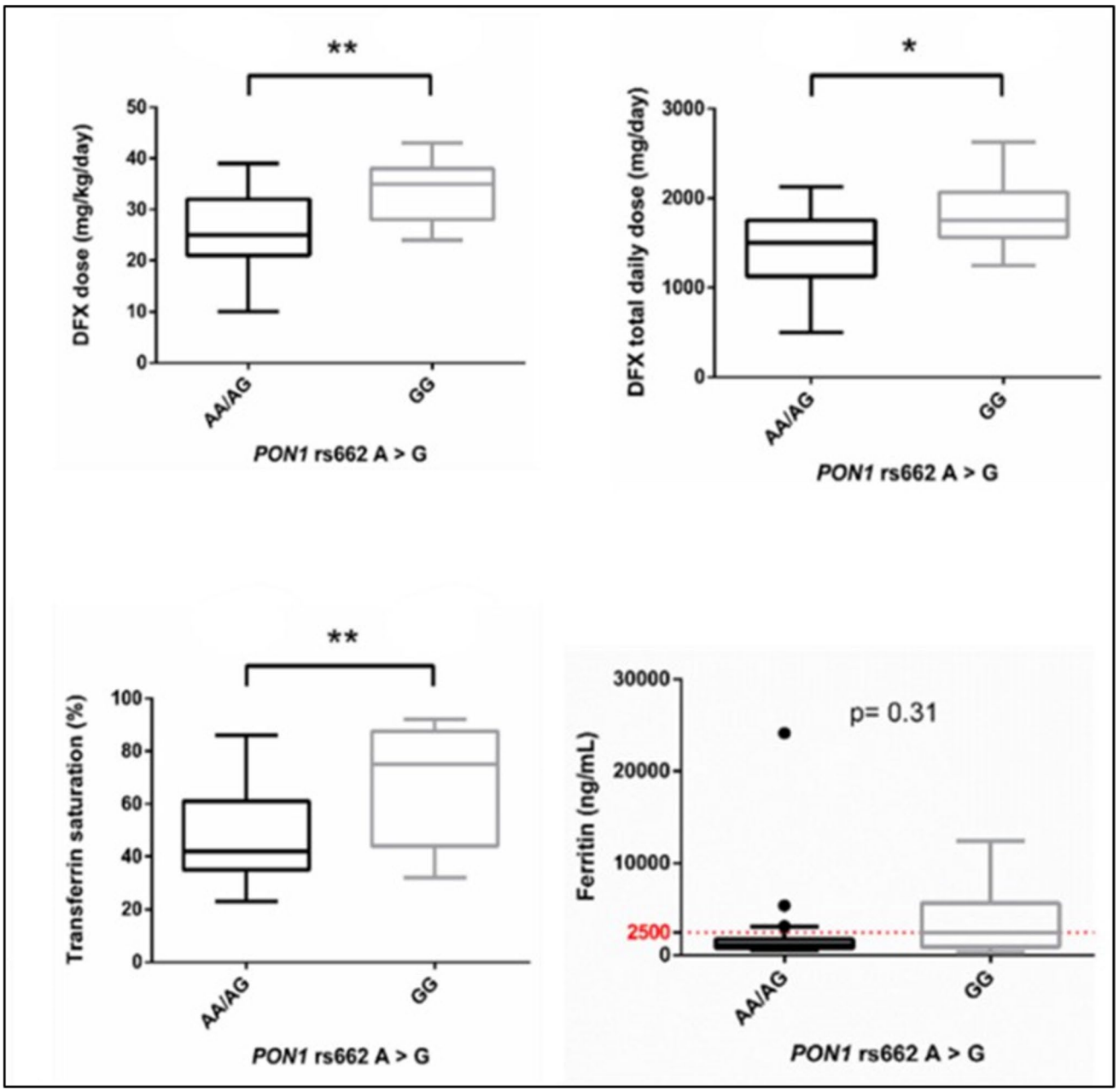

Fig. 2 (continued)

Unit/mL) ($p < 0.001$). In contrast, those who carried *PON-1* rs662 GG had higher transferrin saturation [75 (44–88) vs. 42 (35–61) ($p < 0.01$)], daily total doses of DFX [1750.0 (1562.5–2062.5) vs. 1500.0 (1125.0–1750.0); $p < 0.05$], and daily dose per kilogram values [35.3 (27.9–37.6) vs. 25.0 (21.3–32.3); $p < 0.01$], while lower serum zinc levels were observed [756.7 (557–999) vs. 807.0 (632–1204) ($p > 0.05$)] in *PON-1 GG* carriers. Although no significant difference in serum ferritin (SF) concentrations was observed between patients carrying the *PON-1* or *UGT1A1* variants and other thalassemia major (TM) patients, subgroup statistical analyses were performed to assess the association of these genetic polymorphisms with iron-related parameters, including SF, transferrin saturation, and cardiac iron loading as measured by T2* values. The results of these analyses are summarized in Table 2.

Patients who had an SF level greater than 2500 ng/mL tended to have lower mean zinc levels (mean value, 751.69 ± 118.92; min–max, 557–887 mcg/L) than did those with an SF concentration less than 2500 ng/mL (802.61 ± 128.43 mcg/L). The mean zinc level of those who were considered to have hypozincemia or zinc deficiency

Table 2 Impact of variables on the iron control parameters including serum ferritin, transferrin saturation, and cardiac iron indicator (T2* value)

rs, *n* (%)	Serum ferritin (ng/mL) [median (IQR)]	*p*	Transferrin saturation (%) [median (IQR)]	*p*	Cardiac, T2* (ms) [median (IQR)]	*p*
PON-1 A > G rs662						
AA/AG, 27 (61)	1152.3 (791.3–1731.5)	NS	42 (35–61)	< 0.01*	41.5 (20–57)	NS
GG, 17 (39)	2489.6 (885.8–5659.3)		75 (44–88)		44.0 (28–59)	
UGT1A1 C > T rs887829						
CC, 32 (73)	1178.5 (805.9–2553.7)	0.05	0.5 (0.4–0.8)	NS	44.0 (31.3–60.0)	NS
CT/TT, 12 (27)	1528.1 (832.4–6779.2)		0.4 (0.3–0.8)		39.0 (23.0–55.0)	

NS: $p > 0.05$

IQR interquartile range

was 628.0 ± 41.9 mcg/L (min–max, 557–692). Notably, those patients who were zinc deficient (< 600 mcg/L) had a mean SF concentration as high as 4224.5 ± 2608.8 ng/mL ($n = 3$), and all of these patients had abnormally low vitamin D levels (17.2 ± 1.0 ng/mL).

To compare blood parameters in patients with UGT1A1 rs887829 polymorphism variants, subgroup analysis of patients stratified according to the *UGT1A1* rs887829 polymorphism revealed percentages (%) of 73%, 23%, and 5% for those with wild-type (CC; $n = 32$), heterozygous (CT; $n = 10$), and homozygous (TT; $n = 2$) genotypes, respectively. The mean 25(OH)D levels for each subgroup ranged from 16.5 ± 7.6 to 17.5 ± 7.3 ng/mL in comparison to those who carried TT ($n = 2$), who had a mean vitamin D level of 18.3 ± 0.1 ng/mL. The mean vitamin D concentration among patients with the UGT1A1 CC or CT genotypes (merged group) of *UGT1A1* CC/CT patients was 16.7 ± 7.1 ng/mL ($n = 42$). Four patients (three with the CC genotype and one with the CT genotype) had 25(OH)D levels within the recommended normal range of 30–100 ng/mL, ranging from 30 to 38 ng/mL. Vitamin D deficiency (< 10.0 ng/mL) was observed in only three patients, all of whom carried the CC genotype. When patients were subgrouped on the basis of whether they carried the CC variant or other variants (CT/TT), those with the CC variant had marginally lower median SF levels (1187.5 (range between 805.9 and 2553.7) vs. 1528.1 (832.4–6779.2), *p*-value 0.05). However, there was no significant difference in cardiac T2* among *UGT1A1* rs887829 variants or in the median T2* [44.0 (31.3–60.0) vs. 39.0 (23.0–55.0); $p = 0.39$]. There was also no significant difference in zinc levels among the *UGT1A1* rs887829 variant groups, although there was a marginally lower mean serum zinc level in patients who carried the TT variant than in those who carried the CC/CT variant (620.5 ± 31.8 vs. 795.5 ± 123.8; $p = 0.05$), as shown in Fig. 3. The advance statistical tests approached by multiple logistic regression analysis were performed to evaluate the association between antioxidant measurements, two genetic polymorphisms (PON-1 and UGT1A1), and cardiac iron status, as indicated by T2* values, and are summarized in Table 3. While the associations did not reach statistical significance, the presence of variants in either the *PON-1* or *UGT1A1* genes was associated with increased odds of having lower cardiac T2* values (< 30 ms), with odds ratios of 2.44 and 2.899, respectively. In contrast, higher PON-1 activity appeared to have a protective role against cardiac iron overload.

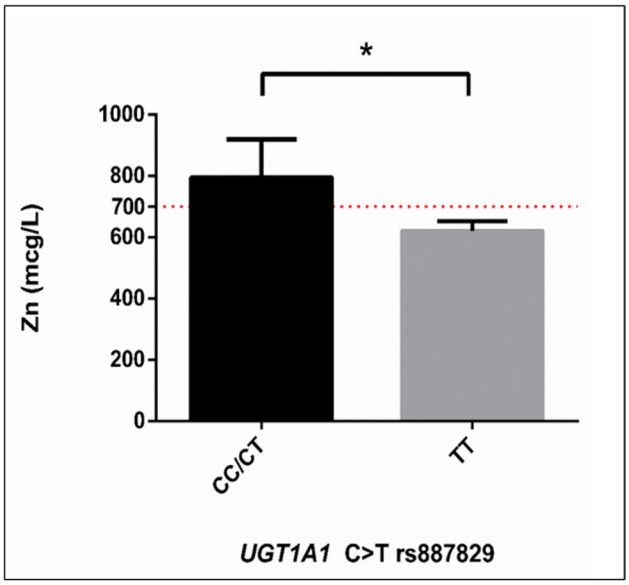

Fig. 3 Comparison of serum zinc levels in thalassemia major (TM) patients stratified by UGT1A1 rs887829 genotypes (CC/CT vs. TT). *$p = 0.05$. Data are presented as mean ± SD, as appropriate

Discussion

Beta-thalassemia is a type of transfusion-dependent anemia, and iron overload is the leading cause of death and morbidity for these patients. The present study is the first to provide evidence that Taiwanese TM patients carrying the *PON-1 GG* or *UGT1A1 TT* genotype have significantly

Table 3 Impact of variables on the cardiac iron indicator (T2* value)

Ind. variable	Odds ratio	5% conf. lower	95% conf. upper
Serum ferritin (ng/mL)	1	1	1
DFX total daily dose (mg/day)	1.002	1	1.005
Zn (mcg/L)	1.001	0.994	1.009
25(OH)D	1.055	0.917	1.215
PON-1 activity	0.804	0.48	1.347
PON-1 rs662 G allele	2.44	0.637	9.349
UGT1A1 rs887829 T allele	2.899	0.674	12.466

lower PON-1 activity. The observation was that lower serum zinc levels were present in both genotypes but reached statistical significance only in patients with the *UGT1A1 TT* genotype and not in those with the *PON-1 GG* genotype. Patients subjected to stable DFX treatment who had moderate iron overload status had significantly lower PON-1 activity and vitamin D levels, particularly those with T2* < 30 ms. Such observations suggest that patients with higher PON-1 activity may have achieved better iron chelation outcomes because they were carrying the appropriate polymorphisms and had an appropriate antioxidant status. In the present study, as shown in Fig. 2, patients who carried PON-1 rs662 with homozygosity for the G allele (GG) had significantly lower PON-1 activity and were prescribed a higher DFX dose per kilogram ($p < 0.01$) and daily total dose ($p < 0.05$). A previous study also revealed that a restored plasma PON-1 level in patients was associated with reduced cardiovascular risk under treatment with another iron chelator and deferiprone, and a possible mechanism involving improvements in oxidative status [28] was also suggested. However, different patterns may be related to ethnicity. Dardiotis et al. [29] suggested that *PON-1* rs662 SNP variants correlate with the activity of PON-1 and that those carrying the A allele have lower PON-1 activity [30], which is different from the current observations.

DFX itself is also known to possess antioxidative activity regarding the oxidation of ascorbic acid with the participation of iron ions and to reduce the reaction rate in vitro [31]; however, the improvements resulting from the combination of vitamin C as an adjuvant therapy for moderately iron-overloaded young vitamin C–deficient patients with TM were not as appreciable as those resulting from deferoxamine [32]. Allelic discrimination results suggest that *UGT1A1* 364 CT/TT and *CYP27B1* 1260 GT/TT positively predict liver stiffness; therefore, alleles such as *CYP2D6* may also influence DFX pharmacokinetics [5] to show individual variation in drug levels even at steady status and thus to provide variability in antioxidant support. For example, the dose used and SF negatively predict T2* data, whereas age and the *CYP2D6* 1457 GG genotype positively influence these values.

In the present study, we did not determine drug levels but focused on an additional role of UGT1A1 involving vitamin D, which is also modulated by zinc ions. As summarized in a review [33], deficiency in vitamin D also further reduces antioxidant capacity because its antioxidant mechanisms are related to its membrane and lipoprotein scavenger activities, which can reduce iron damage, ferroptosis, and ROS production. Therefore, when considering the impacts of *UGT1A1* rs887829, those who carry CT/TT had higher median SF levels (Table 2), possibly resulting from low vitamin D levels. These observations involving the vitamin D pathway provide additional considerations for why the variants observed in Italian patients (*UGT1A1* rs887829 TT) were found to be predictive of ferritin levels [7].

The results of the present study also support this trend because the iron overload status (SF concentration) in patients who carry the *UGT1A1* rs887829 wild type was mostly less than 2500 ng/mL, which may indicate decreased long-term cardiac iron overload risk (Table 2). Moreover, *UGT1A1* rs887829 also had an impact on the trend in zinc level because the mean serum zinc concentration was marginally significantly lower (620.5 ± 31.8 mcg/L) in patients who carried the TT genotype (Fig. 3). Those carrying *UGT1A1* rs887829 CC/CT were considered zinc insufficient (mean value for 795.5 ± 123.8 mcg/L), with a diagnostic cutoff value for zinc deficiency less than 700 mcg/L in Taiwan or with a recommended serum zinc concentration above 800 mcg/L in other countries, such as Japan.

In our previous study, therapeutic drugs were monitored, and the ratio of the parent drug to the chelated metabolite was calculated as an outcome indicator [1]. A plasma DFX-iron complex formation ratio greater than 0.05 was associated with a better iron-control response in patients with DFX. Iron overload is not always well managed by oral iron chelators; therefore, monitoring indicators are needed to identify those who need to receive additional attention and care. The results of the present study may lead to additional biochemical parameters that can assist healthcare providers in easily identifying these patients' comorbidities. For example, the ferritin threshold, which is likely to indicate cardiac hemosiderosis SF levels less than 2027 ng/mL, is 17.5% [35]. Moreover, 82.9% of β-thalassemia patients with

SF levels above 1090 ng/mL may have liver hemosiderosis, regardless of its grade. In the present study, lower vitamin D levels were seen in our recruited patients whose SF concentration was greater than 1000 ng/mL ($14.9 \pm 4.6 < 21.3 \pm 9.3$; $p < 0.01$) and similar result also as described in Napoli et al.'s study [34], even though almost all of the patients had vitamin D deficiency (range = 5.9–38.9 ng/mL).

Erdoğan et al.'s study [11] reported that a higher dose of DFX tends to chelate zinc, while there is an additional free drug available in the circulation. However, our patients who received a higher dose did not have lower mean zinc levels because the mean zinc levels were 736.0 ± 69.0 mcg/L ($n = 5$), 812.6 ± 150.5 mcg/L ($n = 17$), and 780.0 ± 115.8 mcg/L ($n = 22$) in the respective subgroups on the basis of their DFX dose (mg/kg/day), which was either less than or equal to 20, between 20 and 30, or the treated DFX dose was greater than 30, and there were no significant differences ($p = 0.47$) among those groups. In addition, there were no differences in the DFX dose or biochemical parameters between patients who had normal zinc levels and those whose serum zinc concentration was less than 700 mcg/L. When sex differences in zinc supplementation needs were considered, eight of the patients whose serum zinc level was less than 700 mcg/L were female, whereas only one was male. This finding supports the findings of a previous study showing that there is a sex difference in the serum zinc level in TM patients who might be at risk of hypogonadism [16, 36–38], which may be related not only to the outcome of iron chelation [10, 11] but also to dietary habits. Zinc and iron are present together in foods; therefore, minimizing iron intake due to iron overload is also a possible cause of zinc deficiency in TM patients [39, 40]. Zinc, unlike iron, cannot be stored with protein such as ferritin to buffer blood circulation levels, even though the concentration of zinc is much greater within red blood cells than in serum. In a report published in 2016 [41], patients were challenged with zinc at trace levels for short durations, and such levels were quickly exhausted because of the effects of disease symptoms and iron chelation therapy. Moreover, adequate amounts of zinc are suggested to be required for vitamin D regulation and function. This is because the vitamin D receptor is similar to other steroid receptors and can specifically interact with zinc finger proteins [23]. Therefore, maintaining adequate zinc levels, instead of marginally low levels, may prevent complications [41] or growth retardation, hypogonadism, diabetes, or susceptibility to infection [16, 36–38]. Zinc levels of approximately 1000 mcg/L are recommended for patients with COVID-19 or other conditions that impose stress on the body [42]. Therefore, the recommended zinc concentrations for TM need to be discussed.

The limitations of this study are that other genetic variants, such as rs4646903, rs2606345 in *CYP1A1*, rs2231142 in *ABCG2*, rs22776 in *CYP24A1* [4], rs2273697 in *MRP2*, and rs2231142 in *BCRP1* possibly which may influence the iron chelation efficacy observed in Italians [7] and Han Chinese individuals [43], were not examined. Consequently, the potential impact of these genetic variants on iron chelation efficacy cannot be excluded. Further studies examining these variants and investigating potential interactions through genetic interaction analyses are warranted to more comprehensively understand their influence and to strengthen the current findings. In the present study, we also cannot exclude the possible impact of liver dysfunction on vitamin D production/metabolism, as observed in other TM patients [44], because we did not monitor liver enzyme parameters or diet and lifestyle. Therefore, we cannot address whether the abnormally low vitamin D levels ($n = 40$) in these patients were due to hepatic dysfunctions or individual nutrient intake and inadequate light exposure.

Our findings are the first to show that lower PON-1 activity in Taiwanese patients with TM may result from unacceptable iron overload (transferrin saturation > 50) and from the impact of the *PON-1* GG variant. Transferrin was the main predictor of decreased PON-1 activities in TM patients and less pronounced in patients carrying the A allele, similar to a previous study [45]. These iron-overloaded oxidative stress conditions may have been enhanced by insufficient levels of zinc and vitamin D in most of the recruited patients. Taken together, the above findings may be useful for personalized medicine, and the proposed method may be applied to identify patients who do not respond to current iron chelators or other iron overload patients with hereditary hemochromatosis and myelodysplastic syndromes. In addition to low PON-1 activity, which has been associated with an increased risk of major cardiovascular events [46], a meta-analysis in agreement with observations in coronary artery disease revealed that the *PON-1* rs662 polymorphism appears to be associated with a small increase in the risk of ischemic stroke [47]. Although a high level of hepcidin can be a potential marker of severe iron overload in patients with TM [48], even though it is not usually detected in clinical practice, recently, vitamin D deficiency was demonstrated to stimulate calcium transport through the left ventricular–dependent calcium channel, leading to the transport of non-transferrin-bound iron into the myocardium, which may be associated with cardiac iron uptake and ventricular dysfunction in TM patients [49], while high-dose vitamin D_3 reduces circulating hepcidin [50]. Hence, monitoring vitamin D status in TM patients together with PON-1 activity and the levels of zinc involved in one-carbon metabolism may provide additional biochemical pathways linked to cardiac iron control. Moreover, both zinc and vitamin D are not only considered antioxidant-related nutritional indices but are also fundamentally involved in bone absorption/metabolism, immunology, wound healing, and insulin biosynthesis [22]. In the future, additional studies are warranted

to determine how to supplement both zinc and vitamin D with appropriate zinc forms and dosing regimens in patients with TM.

Conclusion

This study suggests a potential relationship between PON-1 activity, genetic variation, and susceptibility to myocardial iron overload in thalassemia major patients and, therefore, provides an explanation for the individual variability following DFX iron chelation therapy for TM patients. All our recruited patients had a median SF of 1320.8 ng/mL and a cardiac T2* value of 44 ms. Although 80% of patients ($n=35$) had a normal zinc level (> 700 mcg/L), zinc levels were marginally significantly lower in patients with the *UGT1A1* TT genotype. Moreover, 90% of patients had vitamin D levels lower than normal at 30 ng/mL. The patients' cardiac iron T2* values also correlated negatively with their SF levels ($r=-0.38$, $p<0.01$). Subgroup analysis demonstrated that the *PON-1* rs662 AA/AG genotypes, the *UGT1A1* rs887829 TT genotype, and antioxidant-related nutritional indices, such as PON-1 activity/zinc or vitamin D, contributed to better transferrin saturation as an iron chelation outcome. These nutritional statuses and nutritional requirements should become a routine assessment in TM patients to closely monitor cardiac health.

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Author Contribution MYL, CSW and THW were responsible for conceiving and designing the study, as well as the data interpretation and preparation of the draft of the manuscript. MYL, WFT, CSW, CSWang, NS, MLL, JHL, WJC, KHL and THW actively participated in the development of the study. MYL, WFT, and CSW extracted the data and take responsibility together with CSWang, NS, and KHL for the integrity of the analyses. MYL, CSW, WJC, KHL and THW participated in the material resources and investigational methods of the study. MYL, CSW, WJC, KHL and THW participated in the critical revision, and all authors gave final approval of the manuscript to be published.

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Data Availability The data cannot be shared openly and the reason is to protect study participant privacy including the genomic information. Only if the data that find supporting the future policy and those will be available from the corresponding author [THW] upon reasonable request.

Declarations

Ethics Approval and Consent to Participate This clinical protocol was approved by the Research Ethics Committee (REC) of National Taiwan University Hospital (NTUH) and was conducted as set out in the protocol (approval ID 201401095RINC).

Consent for Publication Not applicable.

Competing interests The authors declare no competing interests.

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