Construction of an exposure-pathway-phenotype in children with depression due to transfusion-dependent thalassemia: results of (un)supervised machine learning.

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Abstract

Background: Transfusion dependent thalassemia (TDT) patients are treated with continued blood transfusions and show a higher prevalence of depression. TDT with consequent iron overload and inflammation is associated with increased severity of depressive symptoms in TDT children.

Aim of the study: To construct a pathway-phenotype which combines iron overload and neuro-immune biomarkers with depressive symptom subdomains in TDT children.

Methods: We measured iron status parameters (iron, ferritin, transferrin saturation percentage) and inflammatory (interleukin-1β and tumour necrosis factor-α) biomarkers in TDT (n=111) and healthy (n=53) children and analyzed the results using machine learning.

Results: Cluster analysis separated TDT children with depression from those without depression and revealed two depressive subgroups one with low self-esteem and another with increased social-irritability scores. Exploratory factor analysis validated four depressive symptom dimensions as reliable constructs, namely key depressive, physiosomatic, lowered self-esteem and social-irritability dimensions. Partial Least Squares showed that 73.0% of the variance in a latent vector extracted from those four clinical subdomains, immune-inflammatory and iron overload biomarkers was explained by exposure variables including the number of blood transfusions and hospitalizations and use of deferoxamine. The exposure data, iron and immune biomarkers, and symptom subdomains are reflective manifestations of a single latent trait, which shows internal consistency reliability and predictive relevance.

Conclusions: The nomological network combining exposure, pathways and behavioral phenome manifestations provides an index of overall severity and disease risk and, therefore, constitutes a new drug target, indicating that iron overload and immune activation should be targeted to treat depression due to TDT.

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Introduction

Depression is now conceptualized as a neuro-immune disorder with activation of both the immune-inflammatory response system (IRS) and the compensatory immune-regulatory system (CIRS) (Maes and Carvalho, 2018). IRS activation is associated with an increase in interleukin (IL)-1β, tumour necrosis factor (TNF)-α, IL-6, and complement factors and a decrease in negative acute phase reactants such as zinc (Al-Fadhel et al., 2019; Al-Hakeim et al., 2018; Al-Hakeim et al., 2015b; Maes et al., 2012; Maes et al., 1995). CIRS-related abnormalities in depression include elevated IL-4, soluble IL-2 receptor (sIL-2R), sIL-1R antagonist (sIL-1RA), as well as increased production of IL-10 and positive acute-phase proteins (Maes and Carvalho, 2018). CIRS is involved in depression by regulating the primary immune-inflammatory response, thereby preventing an overzealous IRS (Al-Fadhel et al., 2019; Maes and Carvalho, 2018; Martin-Subero et al., 2016). Depression is also accompanied by increased nitro-oxidative stress and lowered antioxidant defenses (Maes et al., 1994; Maes et al., 2011; Maes et al., 1996), which are closely intertwined with activated immune-inflammatory pathways (Moylan et al., 2014).

The onset of depression is associated with different trigger factors that induce immune and nitro-oxidative stress pathways. For example, exposure to psychological stress, including early lifetime trauma, may cause immune activation and oxidative stress, thereby increasing the risk of depression (Kim and Maes, 2003; Moraes et al., 2018). Other exposures which are associated with activated immune-inflammatory and nitro-oxidative pathways and with the onset of depression are treatment with interferon-α (Wichers and Maes, 2004), leaky gut (Köhler et al., 2016; Slyepchenko et al., 2017), leaky gum (Gomes et al., 2018), and lowered levels of omega-3 polyunsaturated fatty acids (Maes et al., 1999). The theory is that different immune (including cytokines and chemokines) and oxidative and nitrosative stress (including aldehydes, advanced oxidation

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products, hypochlorous stress, and nitroso-cysteinyl) products induce neuro-toxicity including effects on synaptic plasticity, synaptic sampling, receptor expression, neurogenesis, apoptosis pathways, and neurotransmission and consequently depression (Maes et al., 2019; Maes and Carvalho, 2018).

Recently, we discovered that exposure to iron overload due to repeated transfusions in children with thalassemia major (β-TM) is associated with increased depression ratings, which, in turn, are associated with activated immune-inflammatory pathways (Al-Hakeim et al., 2020b). Thalassemia major is a genetic disease resulting in a lack or a significant reduction of the β-globin chain causing an imbalance in globin chains that causes red blood cells (RBCs) fragility and subsequent severe anemia (Thein, 2013). Patients with β-TM need lifetime blood transfusions to maintain hemoglobin rates as high as possible and eradicate excessive erythropoiesis (Compernolle et al., 2018). Chronic blood transfusions may cause hemosiderosis, a type of iron overload, which may cause toxicity to most vital organs (Abdulzahra et al., 2011; Al-Hakeim et al., 2015a; Daher et al., 2017). Iron chelation therapy, which is used to reduce iron overload, has harmful consequences on liver, heart, bones, and the immune system (Hammond et al., 2019) with an increased risk to infections (Shah et al., 2019).

Moreover, exposure to elevated body iron may trigger depressive symptoms in young adults (Richardson et al., 2015). Metafratzi et al. (2001) reported that, in patients with transfusion-dependent thalassemia (TDT), increased deposition of iron might be found in the putamen, caudate nucleus, motor and temporal cortex, which are essential hubs coordinating cognitive functions including implicit and explicit memory (Metafratzi et al., 2001). A magnetic resonance imaging study showed higher iron levels in the choroid plexus and red nucleus in patients with TDT (Qiu

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et al., 2014). Furthermore, elevated iron concentrations in the brain may cause increased oxidative stress toxicity (Jomova and Valko, 2011a; Nnah and Wessling-Resnick, 2018).

A previous study showed that the TDT-associated exposome including the number of blood transfusions, iron overload and immune activation, was significantly associated with the clinical diagnosis of depression and severity of depressive symptoms (Al-Hakeim et al., 2020b). In the latter study, the severity of depressive symptoms was estimated using the Children's Depression Inventory (CDI), which records the severity of depressive symptoms in children based on 27-items (Kovacs, 1992). Depressive phenomenology comprises different dimensions including key depressive symptoms (loss of interest, anhedonia, feelings of guilt), a physio-somatic dimension (fatigue, pain, psychosomatic symptoms), impaired social skills (less socialization, social support, and rewarding relationships), and increased irritability (Anderson et al., 2014; Kupferberg et al., 2016; Lewinsohn, 1974; Stringaris et al., 2013). Nevertheless, there are no data whether the TDT-associated exposome is related to these different symptom dimensions in children.

The interconnections between TDT exposure, the iron overload and immune-inflammatory biomarkers (internal exposure and early phenome) and clinical symptoms (late phenome) offer an adequate theoretical background to model pathway phenotypes. **Figure 1** shows the interactions between the genome and exposome (consisting of general as well as specific exposures) ultimately leading to adverse outcome pathways that cause the clinical phenome of depression (symptoms and phenomenology). When applicated to the TDT study of Al-Hakeim et al. (2020), it shows that specific exposure to TDT and blood transfusions induces iron overload and consequent immune activation and that these exposome factors are associated with the clinical phenome of depression (Al-Hakeim et al., 2020b). Recently, we have reviewed how various machine learning techniques, including partial least squares (PLS) analysis, can be used to construct pathway-phenotypes, which

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are higher-order constructs combining information combining the adverse outcome pathways and symptomatology into new pathway-phenotype scores reflecting the phenome of the illness (Al-Hakeim et al., 2020a). Nevertheless, there are no data, whether a pathway-phenotype or an exposure-pathway-phenotype may be constructed reflecting TDT-related depression.

Hence, the present study aims to use machine learning techniques to construct an iron-overload-neuro-immune-depressed pathway-phenotype in TDT children.

Subjects and Methods

Participants

The present study recruited 111 Iraqi β-TM patients and 53 healthy control children (aged 6-12 years from both sexes). The participants were recruited at the Thalassemia Unit, Al-Zahra'a Teaching Hospital, Najaf, Iraq. Specialized pediatricians diagnosed β-TM using the criteria of the 2019 ICD-10-CM Diagnosis (Code D56.1). The diagnosis was made based upon hematological parameters (mainly; hemoglobin <7g/dl and presence of microcytic hypochromic RBCs with anisopoikilocytosis and increase reticulocyte percentage in the blood smear), clinical symptoms (mainly severe anemia, splenectomy, and abnormal bone growth), and by Hb HPLC as measured by using HPLC (VARIANT^{TM} β-Thalassemia Short Program). The sex distribution and age range of the control group was comparable to that of TDT children. All subjects were free of systemic or immune-inflammatory diseases. We excluded patients or controls with splenectomy, systemic diseases such as hypertension, diabetes mellitus, renal failure, or patients with overt inflammation (serum C-reactive protein (CRP) levels > 6mg/l). The exclusion of subjects with serum CRP > 6 mg/L indicates that increased levels of ferritin are probably associated with iron overload rather than with an acute phase response (Kennedy et al., 2004). All patients included show TDT

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involving regular packed RBCs units at 2-4-week intervals depending on the patient's need to maintain Hb level beyond 9 g/dL. The patients were administered Iron-chelating therapy with 25-50 mg/kg/day of deferoxamine mesylate USP (Desferal®) infusion over 8 hours/day at 3-5 times per week depending on the concentration of serum ferritin. Folic acid was given to patients with a deficit of this vitamin to lessen ineffective erythropoiesis. TDT patients were given vitamin C to assist the chelation of Fe to deferoxamine by releasing Fe from the reticuloendothelial system. One-alpha capsules were given to the patients with calcium metabolism disorders caused by a decreased 1-α hydroxylation.

The symptoms of depression in thalassemia patients were measured using the 27 item Children Depression Inventory (CDI) (Kovacs, 1992). The CDI score is validated in children and is one of the most commonly used screening tests for depression in children (Stockings et al., 2015). The diagnosis depressive disorder due to another medical condition (namely TDT) was made according to DSM-5 criteria or when the total CDI score ≥19. Written informed consent was obtained from the patient's first-degree relatives (mother or father). The research protocol was approved by the IRB of the University of Kufa number 712/2019.

Assays

Fasting venous blood was collected in the early morning hours (between 8.00 and 10.00 a.m.). After complete clotting, the samples were centrifuged at 3000 rpm for 10 minutes, and the separated sera were transported into Eppendorf tubes to be stored at -80 °C until analyzed. Serum iron concentration was estimated by the ferrozine method (Linear, Spain) based on the colorimetric principle. Total iron-binding capacity (TIBC) was measured by adding an iron solution to saturate serum transferrin (Tf) with iron, and the unbound iron portion is precipitated with $MgCO_3$ powder.

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The total iron in the supernatant is then determined by the ferrozine method. Serum ferritin was measured by using an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated VIDAS instrument (BioMérieux Co., France). The inter-assay coefficient of variance (CV%) of iron was less than 2.19%, and ferritin was less than 5.70%. Unsaturated Iron binding Capacity (UIBC) was calculated as UIBC = TIBC - serum iron concentration. The transferrin saturation percentage (TS%) was computed as TS% = Iron × 100/TIBC (McLaren et al., 2001). Transferrin concentrations were calculated from the transferrin saturation percentage and serum iron using the formula: Tf (g/L) = serum iron (μM) / (3.98 * TS%). The formula is based on the molecular weight of 79.570 for transferrin and the maximal binding of 2 moles of Fe^{3+} to each mole of transferrin (Kennedy et al., 2004). Serum IL-1β, IL-10, CCL11, and TNF-α levels were measured by commercial ELISA kits supplied by Elabscience, Inc. CA, USA. For samples with highly concentrated biomarkers, we diluted samples as required. The intra-assay CV (precision within-assay) values were less than 7.0%. A latex agglutination kit supplied by Spinreact®, Spain was used to measure serum CRP of all subjects. Serum Cu and Zn were measured by flame atomic absorption spectrophotometer (AA990, PG Instruments Ltd, Leicestershire, UK) after dilution 1:10 with 6% n-butanol diluent before measurement. This method achieved a 30% increase in sensitivity compared to the use of deionized water only due to decrease viscosity and the difference in droplet formation that produces more accurate results (Meret and Henkin, 1971; Twayej et al., 2019).

Statistical analysis

The difference in variables among groups was assessed by analysis of variance (ANOVA), while the Chi-square test was used to calculate the associations among nominal variables.

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Pearson's product-moment and Spearman's rank-order correlation coefficients were used to compute the associations among variables. Multiple regression analysis was used to delineate the significant iron and neuro-immune biomarkers that are associated with the CDI total score. The results were tested for multicollinearity (tolerance and VIF values) and homoscedasticity. Univariate GLM analysis was used to examine the associations between diagnostic classes and biomarkers while adjusting for possible intervening variables. Model-generated estimated marginal mean (SE) values were calculated after correcting for those background variables. Protected LSD tests were used to examine pairwise comparisons among treatment means. Multiple comparisons and correlations were adjusted for false discovery rate (FDR) (Benjamini and Hochberg, 1995). The diagnostic performance was assessed using the area under (AUC) the ROC curve with sensitivity and specificity. The immune and iron biomarkers distributions (tested with the Kolmogorov-Smirnov test) were normalized by processing IL-1β in Ln transformation and CCL11, ferritin, TNF-α, and UIBC in the square root (sqr) transformation. We computed two relevant z-unit weighted composite scores, namely z sqr TNF-α + z Ln IL-1β (zTNFα+IL1β; indicating M1 macrophage activation) and z Iron + z TS% + z Ln ferritin (zFeTSFerr; indicating iron overload). All tests were 2-tailed, and a p-value of 0.05 was used for statistical significance. The above statistical analyses were performed using IBM SPSS windows version 25, 2017.

We performed K-mean, K-median and hierarchical cluster analysis using SPSS 25 and the Unscrambler (CAMO, 2019) in order to find new clusters of participants based on the exposome markers (blood transfusions, iron overload and immune biomarkers). We employed exploratory factor analysis (EFA) using FACTOR, windows version 10.5.03 (Lorenzo-Seva and Ferrando, 2013, 2019) as described previously (Almulla et al., 2020). In order to study the causal associations between exposure variables (iron overload and immune biomarkers and the number of blood

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transfusions and hospitalizations) and the symptomatic depression phenome (the CDI subdimensions), we used partial least squares (PLS) path modelling employing the Smart PLS software (Ringle et al., 2015). Smart PLS uses structural equation modelling to examine the causal paths connecting input indicator variables, or latent vectors (LV) extracted from indicators of external exposure data (TDT) and an output LV extracted from indicator variables reflecting a pathway-phenotype (Ringle et al., 2015). Complete PLS path analysis on 5000 bootstrap samples was conducted only when the inner and outer model constructs complied with specific quality criteria, namely, the model standardized root mean residual (SRMR) < 0.08, and all LVs show suitable reliability validity as indicated by Cronbach's alpha > 0.7, composite reliability > 0.7, rho_A > 0.8, average variance extracted (AVE) > 0.5; and all outer model factors show loadings > 0.6 at $p<0.001$ (Ringle et al., 2015). Consequently, path coefficients with exact p-values are computed using complete PLS with 5000 bootstrap samples.

Results

1. Results of EFA

In order to find meaningful symptom patterns in the CDI items (i.e. the 4 symptom domains described in the "Introduction") and to cluster subjects based on their biomarkers, we performed EFA and cluster analysis, respectively. Firstly, we grouped CDI symptoms in four different symptom subdomains (based on common knowledge, see Introduction) and explored whether these domains may be valid constructs. As such we constructed a first symptom domain using items (1) "I feel sad"; (4) "I do not enjoy anything"; (5) "sometimes I am bad"; (6) "I think something bad will happen to me"; and (10) "every day, I feel like crying" and labelled this symptom domain "key depressive symptoms (KYEDEP)". The second domain comprised 5 items

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namely item (15) “I have to force myself to do my homework”; (16) “I have difficulty falling asleep”; (17) “I always feel tired”; (18) “Most of the days, I don’t have much desire to eat”; (19) “I am always worried about pain and trouble”, labelled as physio-somatic symptoms (PHYSIOSOM). The third domain comprised 6 items namely items (11) “Most often, everything irritates me”; (12) “I don’t like to be around people at all”; (20) “I feel alone all the time”; (22) “I have no friends”; (26) “I never do what I am asked to do”; and (27) “I always fight with people”, and this domain was labelled as “social-irritability symptoms (SIRR)”. The fourth symptom domain comprised 6 items namely items (2) “Everything always happens the way I don’t want them to”; (3) “I always do everything the wrong way”; (7) “I hate myself”; (8) “I am responsible for anything bad that happens to me”; (14) “I am ugly”; and (23) “I am bad at those lessons in which I used to get good grades”, labelled as “lowered self-esteem (LSE)”.

Table 1 shows the results of four different EFAs performed on the 4 symptom domains herein constructed. The Keiser-Meier-Olkin (KMO) statistics as well as the significances of Bartlett's test (all $p<0.00001$) showed that the factorability of the correlation matrices was sufficient to allow EFA being performed. Schwartz’s Bayesian Information Criterion (BIC) and Hull tests indicated that the advised number of factors was one in all four EFAs performed. Table 1 also shows that all CDI items loaded highly on their respective factors. The mean of item residual absolute loadings (MIREAL) (all <0.300) and unidimensional congruence (UNICO) (all >0.95) values suggested that the data may be treated as unidimensional (except UNICO values in self-esteem). The distribution of residuals as assessed with root mean square of residuals (RMSR) performed well, and the weighted root mean square residual (WRMR) and root mean square error (RMSE) values indicated adequate fits of all 4 factors. The factor determinacy index (FDI) values (all >0.80) show that the factor scores have adequate quality and effectiveness. Moreover, the

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comparative fit index (CFI) and goodness of fit index (GFI) indicate an adequate fit of the 4 models. Also, the explained variances of the factors and Cronbach alpha are sufficient.

Results of cluster analysis

K-mean cluster analysis performed on the 4 symptom subdomains showed the existence of three different clusters, whereby one cluster (n=91) comprised all healthy control children (n=53) and 38 TDT children, and two other groups with increased depressive symptoms. Therefore, the data set was divided into 4 different groups, as shown in **Table 2**. This Table shows the four symptom subdomain scores in these four subgroups. There were 2 groups (comprising 32 and 41 children) with meaningful depressive symptoms (thus n=71 with depression due to TDT) both with high scores on the KEYDEP, PHYSIOSOM, LSE, and SIRR domains and their sum. These two depressive classes showed domain scores that were significantly higher than in the 53 healthy children and 38 TDT children without depressive symptoms. Moreover, there are significant differences between the two "depressive" classes in that one class showed significantly increased SIRR scores (labelled as TDT+SIRR), while the other group showed significantly increased LSE scores (labelled TDT+LSE). As such, the cluster analysis detected two different depressive TDT groups, namely depressed TDT children with high LSE scores (TDT+LSE) and depressed children with increased SIRR scores (TDT+SIRR).

Table 3 shows the association between the clinical DSM-5 classification and the cluster analysis-generated solution. There was a highly significant association between both diagnostic classifications (χ^2=247.23, df=6, p<0.001) whereby the cluster depression class (n=73) is enriched with 19 subjects that were classified as non-MDD patients (n=57) using the clinical diagnosis. Those results show that this cluster-generated diagnosis of depression (thus TDT+LSE and

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TDT+SIRR) is less restrictive than the clinical diagnosis of depression. Table 2 shows that the age of TDT+LSE patients was significantly higher than in all other groups while there were no significant differences in sex ratio or urban/rural ratio between the 4 diagnostic groups. TDT+LSE and TDT+SIRR children underwent significantly more blood transfusions and hospitalizations than TDT children. Using the sum of the items in the 4 subdomains showed an AUC ROC curve of 0.998 (SE=0.002; $p<0.001$) with sensitivity of 95.9% and specificity of 100% when using a > 10 cut-off value.

Table 4 shows the difference in iron variables between the 4 study groups. Iron and the integrated index zFeTSFerr score were significantly higher in TDT+LSE than in the three other groups. Overall, TDT patients show signs of iron overload (increased Fe, lowered UIBC, and increased ferritin and the integrated index) as compared with healthy control children. **Table 5** shows the measurements of the immune and mineral variables between the 4 diagnostic groups. IL-1β was significantly higher in TDT+LSE and TDT+SIRR as compared with healthy and TDT children, while TNF-α was significantly higher in TDT+SIRR than in controls and TDT. CCL11 was lower in TDT than in controls while the integrated indices zTNF+zIL1β and (zTNFα+zIL1β)-zIL-10 are significantly higher in TDT+LSE and TDT+SIRR than in healthy control children and TDT. The omnibus tests computed on zinc and copper yielded nonsignificant results. FDR p=correction did not change any of these results.

Associations between symptom domains and biomarkers.

Table 6 shows the results of automatic stepwise regression analyses with the symptom domains as dependent variables and the biomarkers as explanatory variables while allowing for the effects of age, and sex (the latter was never significant in the analyses). Table 6, regression #1

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shows that 37.7% of the variance in the KEYDEP score could be explained by ferritin, zTNF+zIL1, and age (all positively associated). Regression #2 shows that 41.2% of the variance in PHYSIOSOM score could be explained by ferritin, IL-1β, and copper (all positively associated). Regression #3 shows that 48.7% of the variance in LSE scores was explained by iron, age, and ferritin. Regression #4 shows that 39.6% of the variance in the SIRR score was explained by the combined effects of ferritin and zTNF+zIL1. Regression #5 shows a regression with the first PC subtracted from the 4 subdomain PC scores as an explanatory variable. This PC explained 63.39% of the variance in the 4 domain factor scores and showed adequate KMO (0.750) and Bartlett's test (χ^2=224.84, df=6, p<0.001) values. Regression #5 shows that 57.1% of the variance in this PC was explained by ferritin, zTNF+zIL1, age, and TS% (all positively associated). **Figure 2** shows the partial regression of the PC extracted from the 4 subdomains on ferritin after controlling for the effects of sex and age. **Figure 3** shows the partial regression of the same PC on the zTNF+zIL1 index again after controlling for the effects of sex and age. The introduction of the number of blood transfusions in this analysis showed that 64.4% of the variance (F=72.01, df=4/159, p<0.001) was explained by the number of blood transfusions, zTNF+zIL1, and age, while TS% was no longer significant. Finally, we also performed the same regression analyses with the PC extracted from the 4 subdomains as a dependent variable in both healthy and TDT children. In doing so, we observed that in healthy children, 30% of the variance in this PC could be explained by age and copper (both positive), while 19.2% of the variance in TDT was explained by zTNF+zIL1 and transfusions number.

Results of PLS path and factor analysis

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Figure 4 displays the construction of a pathway-phenotype based on the four subdomain scores combined with iron overload status and immune biomarkers. A latent vector (LV) was extracted from these indicator variables in a reflective model and entered in the PLS model as the target variable. This LV loaded highly (all > 0.62 at $p<0.0001$) on all biomarker indicators, except CCL11 and IL-10, and, therefore, we deleted these two immune biomarkers from this LV. We entered an LV extracted from the number of transfusions and hospitalization and use of deferoxamine as an index of iron overload and consequences, labelled TDT LV. The model fit was very good with SRMR of the saturated model = 0.047 and SRMR of the estimated model = 0.050. The construct validities of the pathway-phenotype were very good with Cronbach α= 0.919, composite reliability = 0.931, rho_A = 0.926, and average extracted variance = 0.533, while the loadings of all indicators on this LVs were > 0.620 at $p<0.0001$. Also, the TDT LV showed adequate construct validity with Cronbach α= 0.904, composite reliability = 0.939, rho_A = 0.922, and average extracted variance = 0.837, while the loadings of this LV were > 0.902 at $p<0.0001$. Complete PLS path analysis on 5000 bootstrap samples showed that 73.0% of the variance in the pathway-phenotype LV was explained by the regression on the TDT LV, which also predicted lowered CCL11 and higher IL-10 values. Blindfolding showed that the replicability of the pathway-phenotype LV is adequate with a cross-validated redundancy of 0.377. CTA showed that this model was not mis-specified as a reflective model. Multi-group analysis (MGA) showed that there were no significant differences between boys and girls in the path from TDT LV to the pathway-phenotype LV.

Higher-order constructs using PLS

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Figure 5 shows a second PLS analysis with the construction of multiple higher-order constructs. In brief, we constructed an exposure-pathway-phenotype as a second higher-order construct based on LVs extracted from the symptom phenome (the four symptom dimensions) and the exposome. The latter is a higher-order construct based on an LV extracted from all inflammatory, iron overload, and external exposure indicators. The inflammation LV was extracted from three indicators namely IL-1β, TNF-α, and their integrated index, the iron overload LV was extracted from four indicators namely iron TS%, ferritin, and their integrated index, while the external exposure LV comprised three indicators namely number of transfusion and hospitalizations due to TDT and use of deferoxamine. Most importantly, Figure 5 shows that the composite reliability of all constructs was adequate (shown as figures in the circles delineating the LVs). Likewise, Cronbach alpha (all > 0.877), rho-A (all > 0.809) and AVE (all > 0.625) values were more than adequate. The construct validated redundancies of iron overload (0.407), inflammation (0.373), exposome (0.619), late phenome (0.381), and the exposure-pathway-phenotype (0.567) were more than adequate. Consequently, we have computed the latent variable scores and show these scores in normal controls, TDT, and TDT with depression (LSE and SIRR combined). **Figure 6** shows the bar graph with the latent variable scores in these 3 groups. ANOVA showed significant differences in all 6 scores (all $p<0.001$, at df=2/161). Post-hoc tests showed that all scores were significantly different between the three study groups except iron overload, which was not significantly different between TDT with and without depression ($p=0.052$).

Discussion

The first major finding of this study is that four different dimensions in depressive phenomenology could be validated using EFA, namely key depressive symptoms, physiosomatic

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symptoms, lowered self-esteem and social-irritability. In fact, all TDT children showed increased scores on these four depressive subdomains when compared to healthy children. Furthermore, PLS analysis showed that these four subdomains are reflective manifestations of an underlying construct, which represents the four symptom subdomains and shows adequate construct validity and replicability and is, therefore, a reliable index of overall severity of depression in TDT children.

Some previous studies showed that the majority of patients with thalassemia major have at least one psychiatric disorder (Naderi et al., 2012; Nasiri et al., 2014; Yengil et al., 2014) including anxiety (Maheri et al., 2018; Mednick et al., 2010; Yahia et al., 2013), depression (Maheri et al., 2018; Töret et al., 2018; Yahia et al., 2013), and lack of control of anger (Ghanizadeh et al., 2006). In another study, about half of patients with thalassemia have depression, while 62.7% suffered from irritability and anger (Ghanizadeh et al., 2006). Interestingly, depression and anxiety scores declined in older thalassemia patients (14-58 years old) (Mednick et al., 2010) indicating a difference in the phenome of childhood and later-life depression. Typical depressive symptoms in children with thalassemia are sadness and disinterest in life (Behdani et al., 2015) while compliance with thalassemia treatment may impact self-esteem (Pradhan et al., 2003). It should be added that lowered self-esteem frequently occurs in patients with thalassemia (Goulas et al., 2012). Children with thalassemia are perceived to be more aggressive, not obeying rules, irritable and more challenging while somatization in the form of vague and ill-defined joint pain, body ache, nausea and headaches is frequently observed (Gupta et al., 2012).

Furthermore, our cluster analysis separated depressed TDT patients from healthy children and TDT children without depressive symptoms. It is important to note that our cluster analysis-generated solution of depression due to TDT was more liberal than the clinical diagnosis. Towards

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this end, a total sum score > 10 on 22 selected CDI items may be used to diagnose children with depression due to TDT with a sensitivity of 95.9% and a specificity of 100%. Previous research proposed that a CDI total score of 20 may be used as an external validating criterion with a sensitivity of 83% and a specificity of 89% (Bang et al., 2015). Interestingly, depression is highly prevalent in Iraqi thalassemia patients with a prevalence as high as 75% (Sami Kh and Al-Hamzawi, 2009). Therefore, it is important to authenticate children with depression due to TDT using less-restrictive algorithms with better accuracy. Moreover, this subgroup of depressed TDT children may be further divided into those with lowered self-esteem and those with increased irritability scores. This classification was externally validated by a higher age and iron levels and a higher iron-overload index in children with depression with lowered self-esteem while there were no differences in the number of transfusions between both depressed TDT classes.

The second major finding of this study is that the four depressive symptom domains were predicted by different combinations of biomarkers, whereby key depressive symptoms, physiosomatic symptoms and irritability were predicted by combinations of iron overload and immune-inflammatory biomarkers. In contrast, lowered self-esteem was predicted by iron-overload markers only while increased copper was a significant predictor of increased physiosomatic symptoms. Iron overload is associated with an immune-inflammatory response, including activation of M1 macrophage polarization and increased TNF-α expression (Maras et al., 2018; Wessling-Resnick, 2010a; Zhou et al., 2018). Iron overload may also impact physiosomatic symptoms as indicated by findings that increased liver iron concentrations are accompanied by increased tiredness and fatigue (Abetz et al., 2006; Adams et al., 1997). Moreover, fibromyalgia and depression symptoms may be observed in iron overloaded patients due to hereditary disorders (Mohammad et al., 2013). Diverse immune-inflammatory

biomarkers are associated with the fatigue, physiosomatic and fibromyalgia-like symptoms that accompany depression (Anderson et al., 2014; Maes et al., 2013). Some authors also reported associations between lowered self-esteem and activated immune-inflammatory pathways (Lee and Way, 2019; Opheim et al., 2020).

The mechanisms underpinning the impact of exposure to blood transfusions and chelation treatment on immune-inflammatory pathways and depressive behaviors were reviewed previously and involve iron homeostasis, inflammation, infections, and oxidative stress (Al-Hakeim et al., 2020b; Deb et al., 2009; Enculescu et al., 2017; Ganz, 2018; Wessling-Resnick, 2010b). There is now abundant evidence that activated immune-inflammatory pathways, which are caused by iron overload, may induce these depressive symptom domains (Al-Hakeim et al., 2020b; Leonard and Maes, 2012). Multiple blood transfusions in TDT patients may explain that the immune system is continuously stimulated by alloantigens despite the suppressed immune response due to iron overload (Sari et al., 2016). Moreover, chelation therapy can further increase inflammatory burden by lowering the labile iron pool thereby increasing IL-1β transcription (O'Brien-Ladner et al., 2000). Iron overload in the liver and peripheral blood mononuclear cells induces genes linked to antioxidant activity, and reactive oxygen species, including hydrogen peroxides (Bresgen and Eckl, 2015). The combination of iron overload, and neuro-immune and oxidative stress toxicity following exposure to blood transfusions and deferoxamine treatment may cause neurotoxic effects leading to the development of various symptoms, including depression (Jomova and Valko, 2011b; Nnah and Wessling-Resnick, 2018; Siesjö et al., 1989). Both inflammation and oxidative stress may explain depression symptoms (Hirose et al., 2016), including physiosomatic symptoms (Chaves-Filho et al., 2019), low self-esteem (Bhatt et al., 2020) and irritability (Maria Michel et al., 2012).

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The third significant finding of this study is that a latent vector could be extracted from the four clinical subdomains, and the iron overload and immune-inflammatory biomarkers. As such, we have constructed a pathway-phenotype that combines changes in biomarkers (early phenome features) with specific symptom domains (late phenome features) into an index reflecting the phenome of depression due to TDT. The latter provides an index of overall severity of chemical stress and phenomenology and follows a reflective model with excellent construct validity indicating that all those indicators are reflective manifestations of a common underlying construct, namely the core phenome of depression. Importantly, our pathway analysis showed that a large part of the variance in this phenotype-pathway was explained by the exposure to TDT and its consequences. More specifically, the number of transfusions, hospitalizations and use of deferoxamine is part of the external exposome, which may cause adverse outcome pathways including iron overload as well as activated immune-inflammatory pathways and, consequently, adverse health outcomes, namely the four depressive subdomains which together shape the nosological entity “depression due to TDT”. Complementing the exposome with the adverse outcome pathways and adverse health outcome concepts may promote the mechanistic understanding of exposure-induced effects on pathways and behavior (Escher et al., 2017).

Our second PLS analysis showed that a reliable and replicable latent vector could be extracted from the exposure data, iron overload and immune biomarkers, and symptom domain as well. Therefore, this nomological network combining exposure, pathways and the behavioral phenome into a higher-order, overarching construct indicates that the exposome comprising TDT and the induced chemical stress may induce AHO leading to AHO. This nomothetic approach provides an index of the overall impact of exposure + pathways + phenomenology and consequently of disease risk and severity and, therefore, this index should be regarded as a new

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drug target, indicating that treatments of depression due to TDT should target iron overload and immune activation (and related nitro-oxidative stress toxicity).

This study would have been more interesting if we had assayed nitro-oxidative stress biomarkers, which probably play an essential role in the onset of TDT-related depression. In addition, we did not measure the psycho-social exposure factors, namely difficulties in all facets of life including maladaptive coping strategies, low school or play performance, social isolation, dependency, compromised physical ability and limited life opportunities (Koutelekos and Haliasos, 2013).

Conclusions

TDT children with depression may be discriminated from those without depression using a total sum score > 10 on 22 selected items with a sensitivity of 95.9% and a specificity of 100%. Four depressive symptom dimensions (key depressive, physiosomatic, lowered self-esteem and social-irritability) were validated as reliable constructs and manifestations of an underlying single trait, namely overall severity of depression due to TDT. We found that 73.0% of the variance in a latent vector extracted from those four dimensions, iron overload and immune-inflammatory biomarkers was explained by the number of blood transfusions and hospitalizations and use of deferoxamine.

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Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author's contributions

All the contributing authors have participated in the preparation of the manuscript.

References

Abdulzahra, M.S., Al-Hakeim, H.K., Ridha, M.M., 2011. Study of the effect of iron overload on the function of endocrine glands in male thalassemia patients. Asian J Transfus Sci 5, 127-131.
Abetz, L., Baladi, J.F., Jones, P., Rofail, D., 2006. The impact of iron overload and its treatment on quality of life: results from a literature review. Health Qual Life Outcomes 4, 73.
Adams, P.C., Deugnier, Y., Moirand, R., Brissot, P., 1997. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatology 25, 162-166.
Al-Fadhel, S.Z., Al-Hakeim, H.K., Al-Dujaili, A.H., Maes, M., 2019. IL-10 is associated with increased mu-opioid receptor levels in major depressive disorder. Eur Psychiatry 57, 46-51.
Al-Hakeim, H.K., Al-Khakani, M.M., Al-Kindi, M.A., 2015a. Correlation of hepcidin level with insulin resistance and endocrine glands function in major thalassemia. Adv Clin Exp Med 24, 69-78.
Al-Hakeim, H.K., Al-Kufi, S.N., Al-Dujaili, A.H., Maes, M., 2018. Serum Interleukin Levels and Insulin Resistance in Major Depressive Disorder. CNS Neurol Disord Drug Targets 17, 618-625.
Al-Hakeim, H.K., Al-Rammahi, D.A., Al-Dujaili, A.H., 2015b. IL-6, IL-18, sIL-2R, and TNFα proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation. J Affect Disord 182, 106-114.
Al-Hakeim, H.K., Almulla, A.F., Maes, M., 2020a. The Neuroimmune and Neurotoxic Fingerprint of Major Neurocognitive Psychosis or Deficit Schizophrenia: a Supervised Machine Learning Study. Neurotoxicity Research 37, 753-771.
Al-Hakeim, H.K., Najm, A.H., Al-Dujaili, A.H., Maes, M., 2020b. Major Depression in Children with Transfusion-Dependent Thalassemia Is Strongly Associated with the Combined Effects of Blood Transfusion Rate, Iron Overload, and Increased Pro-inflammatory Cytokines. Neurotox Res 38, 228-241.

Almulla, A.F., Al-Hakeim, H.K., Maes, M., 2020. Schizophrenia phenomenology revisited: positive and negative symptoms are strongly related reflective manifestations of an underlying single trait indicating overall severity of schizophrenia. CNS Spectrums, 1-10.
Anderson, G., Berk, M., Maes, M., 2014. Biological phenotypes underpin the physio-somatic symptoms of somatization, depression, and chronic fatigue syndrome. Acta psychiatrica Scandinavica 129, 83-97.
Bang, Y.R., Park, J.H., Kim, S.H., 2015. Cut-Off Scores of the Children's Depression Inventory for Screening and Rating Severity in Korean Adolescents. Psychiatry investigation 12, 23-28.
Behdani, F., Badiee, Z., Hebrani, P., Moharreri, F., Badiee, A.H., Hajivosugh, N., Rostami, Z., Akhavanrezayat, A., 2015. Psychological Aspects in Children and Adolescents With Major Thalassemia: A Case-Control Study. Iran J Pediatr 25, e322.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological) 57, 289-300.
Bhatt, S., Nagappa, A.N., Patil, C.R., 2020. Role of oxidative stress in depression. Drug Discovery Today.
Bresgen, N., Eckl, P.M., 2015. Oxidative stress and the homeodynamics of iron metabolism. Biomolecules 5, 808-847.
CAMO, 2019. The Unscrambler Appendices: Method References, 2019. . As assessed 19-3-2019. www.camo.com/helpdocs/The_Unscrambler_Method_References.pdf. .
Chaves-Filho, A.J.M., Macedo, D.S., de Lucena, D.F., Maes, M., 2019. Shared microglial mechanisms underpinning depression and chronic fatigue syndrome and their comorbidities. Behav Brain Res 372, 111975.
Compernolle, V., Chou, S.T., Tanael, S., Savage, W., Howard, J., Josephson, C.D., Odame, I., Hogan, C., Denomme, G., Shehata, N., 2018. Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. Transfusion 58, 1555-1566.
Daher, R., Manceau, H., Karim, Z., 2017. Iron metabolism and the role of the iron-regulating hormone hepcidin in health and disease. La Presse Médicale 46, e272-e278.
Deb, S., Johnson, E.E., Robalinho-Teixeira, R.L., Wessling-Resnick, M., 2009. Modulation of intracellular iron levels by oxidative stress implicates a novel role for iron in signal transduction. Biometals 22, 855-862.
Enculescu, M., Metzendorf, C., Sparla, R., Hahnel, M., Bode, J., Muckenthaler, M.U., Legewie, S., 2017. Modelling Systemic Iron Regulation during Dietary Iron Overload and Acute Inflammation: Role of Hepcidin-Independent Mechanisms. PLoS Comput Biol 13, e1005322.
Escher, B.I., Hackermüller, J., Polte, T., Scholz, S., Aigner, A., Altenburger, R., Böhme, A., Bopp, S.K., Brack, W., Busch, W., Chadeau-Hyam, M., Covaci, A., Eisenträger, A., Galligan, J.J., Garcia-Reyero, N., Hartung, T., Hein, M., Herberth, G., Jahnke, A., Kleinjans, J., Klüver, N., Krauss, M., Lamoree, M., Lehmann, I., Luckenbach, T., Miller, G.W., Müller, A., Phillips, D.H., Reemtsma, T., Rolle-Kampczyk, U., Schüürmann, G., Schwikowski, B., Tan, Y.-M., Trump, S., Walter-Rohde, S., Wambaugh, J.F., 2017. From the exposome to mechanistic understanding of chemical-induced adverse effects. Environment international 99, 97-106.
Ganz, T., 2018. Iron and infection. Int J Hematol 107, 7-15.
Ghanizadeh, A., Khajavian, S., Ashkani, H., 2006. Prevalence of psychiatric disorders, depression, and suicidal behavior in child and adolescent with thalassemia major. J Pediatr Hematol Oncol 28, 781-784.
Gomes, C., Martinho, F.C., Barbosa, D.S., Antunes, L.S., Póvoa, H.C.C., Baltus, T.H.L., Morelli, N.R., Vargas, H.O., Nunes, S.O.V., Anderson, G., Maes, M., 2018. Increased Root Canal Endotoxin Levels are Associated with Chronic Apical Periodontitis, Increased Oxidative and Nitrosative Stress, Major Depression, Severity of Depression, and a Lowered Quality of Life. Molecular neurobiology 55, 2814-2827.

Goulas, V., Kourakli-Symeonidis, A., Camoutsis, C., 2012. Comparative effects of three iron chelation therapies on the quality of life of greek patients with homozygous transfusion-dependent Beta-thalassemia. ISRN hematology 2012.
Gupta, V., Singh, A., Upadhyay, S.K., Bhatia, B., 2012. Psychopathology in children with thalassemia major. Psychological studies 57, 55-57.
Hammond, J., Thompson, A.A., Fogel, M.A., Hammond, K., Kokroko, J., Kwiatkowski, J.L., 2019. Combination Oral Chelation in Adult Patients With Transfusion-dependent Thalassemia and High Iron Burden. Journal of pediatric hematology/oncology 41, e47-e50.
Hirose, A., Terauchi, M., Akiyoshi, M., Owa, Y., Kato, K., Kubota, T., 2016. Depressive symptoms are associated with oxidative stress in middle-aged women: a cross-sectional study. BioPsychoSocial Medicine 10, 12.
Jomova, K., Valko, M., 2011a. Advances in metal-induced oxidative stress and human disease. Toxicology 283, 65-87.
Jomova, K., Valko, M., 2011b. Importance of iron chelation in free radical-induced oxidative stress and human disease. Current pharmaceutical design 17, 3460-3473.
Kennedy, A., Kohn, M., Lammi, A., Clarke, S., 2004. Iron status and haematological changes in adolescent female inpatients with anorexia nervosa. Journal of paediatrics and child health 40, 430-432.
Kim, Y.K., Maes, M., 2003. The role of the cytokine network in psychological stress. Acta neuropsychiatrica 15, 148-155.
Köhler, C.A., Maes, M., Slyepchenko, A., Berk, M., Solmi, M., Lanctôt, K.L., Carvalho, A.F., 2016. The Gut-Brain Axis, Including the Microbiome, Leaky Gut and Bacterial Translocation: Mechanisms and Pathophysiological Role in Alzheimer's Disease. Current pharmaceutical design 22, 6152-6166.
Koutelekos, J., Haliasos, N., 2013. Depression and Thalassemia in children, adolescents and adults. Health Science Journal 7, 239.
Kovacs, M., 1992. Children's Depression Inventory Manual. Multi-Health Systems. Inc. North Tonawanda, NY.
Kupferberg, A., Bicks, L., Hasler, G., 2016. Social functioning in major depressive disorder. Neuroscience & Biobehavioral Reviews 69, 313-332.
Lee, D.S., Way, B.M., 2019. Perceived social support and chronic inflammation: The moderating role of self-esteem. Health Psychol 38, 563-566.
Leonard, B., Maes, M., 2012. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neuroscience & Biobehavioral Reviews 36, 764-785.
Lewinsohn, P.M., 1974. A behavioral approach to depression. Essential papers on depression, 150-172.
Lorenzo-Seva, U., Ferrando, P.J., 2013. Factor 9.2: A comprehensive program for fitting exploratory and semiconfirmatory factor analysis and IRT models. Applied Psychological Measurement.
Lorenzo-Seva, U., Ferrando, P.J., 2019. A general approach for fitting pure exploratory bifactor models. Multivariate behavioral research 54, 15-30.
Maes, M., Berk, M., Goehler, L., Song, C., Anderson, G., Gałecki, P., Leonard, B., 2012. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. BMC medicine 10, 66.
Maes, M., Bonifacio, K.L., Morelli, N.R., Vargas, H.O., Barbosa, D.S., Carvalho, A.F., Nunes, S.O.V., 2019. Major differences in neurooxidative and neuronitrosative stress pathways between major depressive disorder and types I and II bipolar disorder. Molecular neurobiology 56, 141-156.
Maes, M., Carvalho, A.F., 2018. The Compensatory Immune-Regulatory Reflex System (CIRS) in Depression and Bipolar Disorder. Molecular neurobiology 55, 8885-8903.
Maes, M., Christophe, A., Delanghe, J., Altamura, C., Neels, H., Meltzer, H.Y., 1999. Lowered omega3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients. Psychiatry Res 85, 275-291.

Maes, M., Delanghe, J., Meltzer, H.Y., Scharpé, S., D'Hondt, P., Cosyns, P., 1994. Lower degree of esterification of serum cholesterol in depression: relevance for depression and suicide research. Acta psychiatrica Scandinavica 90, 252-258.
Maes, M., Galecki, P., Chang, Y.S., Berk, M., 2011. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog Neuropsychopharmacol Biol Psychiatry 35, 676-692.
Maes, M., Meltzer, H.Y., Bosmans, E., Bergmans, R., Vandoolaeghe, E., Ranjan, R., Desnyder, R., 1995. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. Journal of affective disorders 34, 301-309.
Maes, M., Ringel, K., Kubera, M., Anderson, G., Morris, G., Galecki, P., Geffard, M., 2013. In myalgic encephalomyelitis/chronic fatigue syndrome, increased autoimmune activity against 5-HT is associated with immuno-inflammatory pathways and bacterial translocation. J Affect Disord 150, 223-230.
Maes, M., Smith, R., Christophe, A., Cosyns, P., Desnyder, R., Meltzer, H., 1996. Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and increased C20: 4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. J Affect Disord 38, 35-46.
Maheri, A., Sadeghi, R., Shojaeizadeh, D., Tol, A., Yaseri, M., Rohban, A., 2018. Depression, Anxiety, and Perceived Social Support among Adults with Beta-Thalassemia Major: Cross-Sectional Study. Korean J Fam Med 39, 101-107.
Maras, J.S., Das, S., Sharma, S., Sukriti, S., Vyas, A.K., Kumar, D., Bhat, A., Yadav, G., Choudhary, M.C., Sharma, S., 2018. Iron-overload triggers ADAM-17 mediated inflammation in severe alcoholic hepatitis. Scientific reports 8, 1-14.
Maria Michel, T., Pulschen, D., Thome, J., 2012. The role of oxidative stress in depressive disorders. Current pharmaceutical design 18, 5890-5899.
Martin-Subero, M., Anderson, G., Kanchanatawan, B., Berk, M., Maes, M., 2016. Comorbidity between depression and inflammatory bowel disease explained by immune-inflammatory, oxidative, and nitrosative stress; tryptophan catabolite; and gut–brain pathways. CNS spectrums 21, 184-198.
Mednick, L., Yu, S., Trachtenberg, F., Xu, Y., Kleinert, D.A., Giardina, P.J., Kwiatkowski, J.L., Foote, D., Thayalasuthan, V., Porter, J.B., Thompson, A.A., Schilling, L., Quinn, C.T., Neufeld, E.J., Yamashita, R., 2010. Symptoms of depression and anxiety in patients with thalassemia: prevalence and correlates in the thalassemia longitudinal cohort. Am J Hematol 85, 802-805.
Meret, S., Henkin, R., 1971. Simultaneous direct estimation by atomic absorption spectrophotometry of copper and zinc in serum, urine, and cerebrospinal fluid. Clinical Chemistry 17, 369-373.
Metafratzi, Z., Argyropoulou, M., Kiortsis, D., Tsampoulas, C., Chaliassos, N., Efremidis, S., 2001. T 2 relaxation rate of basal ganglia and cortex in patients with β-thalassaemia major. The British journal of radiology 74, 407-410.
Mohammad, A., Carey, J.J., Storan, E., Scarry, M., Coughlan, R.J., Lee, J.M., 2013. High prevalence of fibromyalgia in patients with HFE-related hereditary hemochromatosis. J Clin Gastroenterol 47, 559-564.
Moraes, J.B., Maes, M., Roomruangwong, C., Bonifacio, K.L., Barbosa, D.S., Vargas, H.O., Anderson, G., Kubera, M., Carvalho, A.F., Nunes, S.O.V., 2018. In major affective disorders, early life trauma predict increased nitro-oxidative stress, lipid peroxidation and protein oxidation and recurrence of major affective disorders, suicidal behaviors and a lowered quality of life. Metabolic brain disease 33, 1081-1096.
Moylan, S., Berk, M., Dean, O.M., Samuni, Y., Williams, L.J., O'Neil, A., Hayley, A.C., Pasco, J.A., Anderson, G., Jacka, F.N., Maes, M., 2014. Oxidative & nitrosative stress in depression: why so much stress? Neurosci Biobehav Rev 45, 46-62.
Naderi, M., Hormozi, M.R., Ashrafi, M., Emamdadi, A., 2012. Evaluation of Mental Health and Related Factors among Patients with Beta-thalassemia Major in South East of Iran. Iranian journal of psychiatry 7, 47-51.

Nasiri, M., Hosseini, H., Shahmohammadi, S., 2014. Mental health status in patients with Thalassemia major in Iran. Journal of Pediatrics Review 2, 55-61.
Nnah, I.C., Wessling-Resnick, M., 2018. Brain iron homeostasis: a focus on microglial iron. Pharmaceuticals 11, 129.
O'Brien-Ladner, A.R., Nelson, S.R., Murphy, W.J., Blumer, B.M., Wesselius, L.J., 2000. Iron Is a Regulatory Component of Human IL-1 β Production: Support for Regional Variability in the Lung. American journal of respiratory cell and molecular biology 23, 112-119.
Opheim, R., Moum, B., Grimstad, B.T., Jahnsen, J., Prytz Berset, I., Hovde, Ø., Huppertz-Hauss, G., Bernklev, T., Jelsness-Jørgensen, L.P., 2020. Self-esteem in patients with inflammatory bowel disease. Qual Life Res.
Pradhan, P.V., Shah, H., Rao, P., Ashturkar, D., Ghaisas, P., 2003. Psychopathology and self-esteem in chronic illness. Indian J Pediatr 70, 135-138.
Qiu, D., Chan, G.-F., Chu, J., Chan, Q., Ha, S.-Y., Moseley, M., Khong, P.-L., 2014. MR quantitative susceptibility imaging for the evaluation of iron loading in the brains of patients with β-thalassemia major. American Journal of Neuroradiology 35, 1085-1090.
Richardson, A.C., Heath, A.-L.M., Haszard, J.J., Polak, M.A., Houghton, L.A., Conner, T.S., 2015. Higher body iron is associated with greater depression symptoms among young adult men but not women: observational data from the daily life study. Nutrients 7, 6055-6072.
Ringle, C., Wende, S., Becker, J.J.R.f.h.w.s.c., 2015. SmartPLS 3 [computer software].
Sami Kh, S., Al-Hamzawi, A., 2009. Psychoneurotic Profiles Of Thalassemia. Al-Qadisiyah Medical Journal 5, 23-38.
Sari, T.T., Gatot, D., Akib, A.A., Bardosono, S., Hadinegoro, S.R., Harahap, A.R., Idjradinata, P.S., 2016. Immune response of thalassemia major patients in Indonesia with and without splenectomy. Acta Medica Indonesiana 46.
Shah, F.T., Sayani, F., Trompeter, S., Drasar, E., Piga, A., 2019. Challenges of blood transfusions in β-thalassemia. Blood reviews, 100588.
Siesjö, B., Agardh, C.-D., Bengtsson, F., 1989. Free radicals and brain damage. Cerebrovascular and brain metabolism reviews 1, 165-211.
Slyepchenko, A., Maes, M., Jacka, F.N., Köhler, C.A., Barichello, T., McIntyre, R.S., Berk, M., Grande, I., Foster, J.A., Vieta, E., Carvalho, A.F., 2017. Gut Microbiota, Bacterial Translocation, and Interactions with Diet: Pathophysiological Links between Major Depressive Disorder and Non-Communicable Medical Comorbidities. Psychotherapy and psychosomatics 86, 31-46.
Stockings, E., Degenhardt, L., Lee, Y.Y., Mihalopoulos, C., Liu, A., Hobbs, M., Patton, G., 2015. Symptom screening scales for detecting major depressive disorder in children and adolescents: a systematic review and meta-analysis of reliability, validity and diagnostic utility. Journal of affective disorders 174, 447-463.
Stringaris, A., Maughan, B., Copeland, W.S., Costello, E.J., Angold, A., 2013. Irritable mood as a symptom of depression in youth: prevalence, developmental, and clinical correlates in the Great Smoky Mountains Study. Journal of the American Academy of Child & Adolescent Psychiatry 52, 831-840.
Thein, S.L.J.C.S.H.p.i.m., 2013. The molecular basis of β-thalassemia. 3, a011700.
Töret, E., Karadaş, N., Gökçe, N., Kaygusuz, A., Karapınar, T.H., Oymak, Y., Gözmen, S., 2018. Quality of Life and Depression in Turkish Patients with β-Thalassemia Major: A Cross-Sectional Study. Hemoglobin 42, 326-329.
Twayej, A.J., Al-Hakeim, H.K., Al-Dujaili, A.H., Maes, M., 2019. Lowered zinc and copper levels in drug-naïve patients with major depression: Effects of antidepressants, ketoprofen and immune activation. The World Journal of Biological Psychiatry, 1-12.
Wessling-Resnick, M., 2010a. Iron homeostasis and the inflammatory response. Annual review of nutrition 30, 105-122.

 doi:10.20944/preprints202006.0202.v1

Wessling-Resnick, M., 2010b. Iron homeostasis and the inflammatory response. Annu Rev Nutr 30, 105-122.
Wichers, M.C., Maes, M., 2004. The role of indoleamine 2,3-dioxygenase (IDO) in the pathophysiology of interferon-alpha-induced depression. J Psychiatry Neurosci 29, 11-17.
Yahia, S., El-Hadidy, M.A., El-Gilany, A.H., Anwar, R., Darwish, A., Mansour, A.K., 2013. Predictors of anxiety and depression in Egyptian thalassemic patients: a single center study. Int J Hematol 97, 604-609.
Yengil, E., Acipayam, C., Kokacya, M.H., Kurhan, F., Oktay, G., Ozer, C., 2014. Anxiety, depression and quality of life in patients with beta thalassemia major and their caregivers. Int J Clin Exp Med 7, 2165-2172.
Zhou, Y., Que, K.T., Zhang, Z., Yi, Z.J., Zhao, P.X., You, Y., Gong, J.P., Liu, Z.J., 2018. Iron overloaded polarizes macrophage to proinflammation phenotype through ROS/acetyl-p53 pathway. Cancer medicine 7, 4012-4022.

Table 1: Results of Explanatory Factor Analysis (EFA) performed on selected Children's Depression Inventory items in 164 children.

Features	**Key Depression**		**Physiosomatic**		**Social-irritability**		**Self-esteem**	
Items / Factor Loadings	**Items**	**Loadings**	**Items**	**Loadings**	**Items**	**Loadings**	**Items**	**Loadings**
	1	0.664	15	0.505	11	0.643	2	0.822
	4	0.866	16	0.510	12	0.651	3	0.872
	5	0.636	17	0.531	20	0.521	7	0.682
	6	0.484	18	0.732	22	0.591	8	0.571
	10	0.544	19	0.608	26	0.540	14	0.781
	-	-	-	-	27	0.780	23	0.498
EFA model quality data								
Keiser-Meier-Olkin test	0.759		0.733		0.729		0.781	
Root mean Square Error	0.000		0.042		0.032		0.021	
Root Mean square of residuals	0.0476 (0.0783)		0.0663 (0.0783)		0.0783 (0.090)		0.0706 (0.0783)	
Weighted Root Mean Square Residuals	0.0312		0.0540		0.0575		0.0494	
Comparative Fit index	1.016		0.987		0.994		0.999	
Goodness of Fit Index	1.000		0.992		0.997		1.000	
Explained variance	0.524		0.465		0.485		0.577	
Factor determinacy index	0.916		0.860		0.901		0.946	
Cronbach alpha	0.767		0.710		0.784		0.849	
Unidimensional Congruence	0.984		0.952		0.967		0.916	
MIREAL	0.226		0.252		0.225		0.235	

MIREAL: Mean of Item Residual Absolute Loadings.

Table 2: Differences in symptom subdomains, socio-demographic, and clinical data between 4 diagnostic groups.

Variables	HCC [A] N= 53	TDT [B] N= 38	TDT+LSE [C] N= 32	TDT+SIRR [D] N=41	F/χ^2	df	P
Key depression*	0.65 (0.15) [B,C,D]	1.62 (0.18) [A,C,D]	3.55 (0.22) [A,B]	3.55 (0.18) [A,B]	72.81	3/158	<0.001
Physio-somatic*	1.10 (0.18) [B,C,D]	3.11 (0.22) [A,C,D]	4.04 (0.26) [A,B,D]	4.93 (0.22) [A,B,C]	71.80	3/158	<0.001
Self-esteem*	0.43 (0.15) [B,C,D]	1.85 (0.18) [A,C,D]	5.15 (0.22) [A,B]	1.88 (0.18) [A,B]	109.71	3/158	<0.001
Irritability*	0.93 (0.17) [B,C,D]	1.81 (0.20) [A,C,D]	4.07 (0.24) [A,B,D]	5.48 (0.20) [A,B,C]	125.45	3/158	<0.001
CDI total 22 items*	3.10 (0.37) [B,C,D]	8.37 (0.44) [A,C,D]	16.79 (0.53) [A,B,D]	15.83 (0.44) [A,B,C]	252.78	3/158	<0.001
Age (years)	8.9 (1.9) [C,D]	8.3 (2.1) [C]	10.9 (1.5) [A,B,D]	7.6 (1.9) [A,C]	19.70	3/160	<0.001
Sex (M/F)	27/26	19/19	16/16	23 / 18	0.41	3	0.938
Rural/Urban	10/43	10/28	12/20	12/29	3.70	3	0.296
# Transfusions	-	60.0 (26.2) [C,D]	84.6 (36.9) [A]	87.3 (40.9) [A]	6.94	2/108	<0.001
# Hospitalizations	-	7.4 (4.4)	9.9 (5.0)	8.9 (4.5)	2.60	2/108	0.079

All results are shown as mean (SE) after covarying for age and sex. Other results are shown as mean (SD).

HCC: healthy control children, TDT: transfusion-dependent thalassemia, TDT+LSE: TDT with depression and low self-esteem, TDT+SIRR: TDT with depression and social-irritability

CDI: Children depression inventory scale, sum of 22 selected items

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Table 3: Associations between the clinical diagnosis of depression and the cluster analysis-generated solution

Diagnosis	HCC	TDT	TDT+LSE	TDT+SIRR	Total
HC	53	0	0	0	53
TDT	0	38	11	8	57
TDT+MDD	0	0	21	33	54
Total	53	38	32	41	164

Clinical diagnosis: HCC: healthy control children, TDT: transfusion-dependent thalassemia group, TDT+MDD: depression due to TDT.

Cluster analysis-generated: TDT+LSE: TDT with depression and low self-esteem, TDT+SIRR: TDT with depression and social-irritability.

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Table 4: Differences in iron metabolism biomarkers between healthy control children (HCC), transfusion-dependent-thalassemia (TDT), and TDT with and without depressive symptom domains including lowered self-esteem and social-irritability.

Variables (z scores)	HCC[A]	TDT[B]	TDT+LSE[C]	TDT+SIRR[D]	F	df	P
Iron	-0.907 (0.105) [B,C,D]	0.297 (0.125) [A,C]	0.838 (0.151) [A,B,D]	0.243 (0.126) [A,C]	40.19	3/158	<0.001
UIBC	0.690 (0121) [B,C,D]	-0.220 (0.144) [A]	-0.470 (0.174) [A]	-0.321 (0.146) [A]	16.38	3/158	<0.001
TS%	-0.952 (0.103) [B,C,D]	0.309 (0.123) [A,C]	0.700 (0.148) [A,B]	0.398 (0.124) [A]	43.51	3/158	<0.001
Tf	-0.212 (0.135) [c]	0.006 (0.161)	0.339 (0.194) [A]	0.003 (0.162)	1.88	3/158	0.135
Ferritin	-1.244 (0.070) [B,C,D]	0.454 (0.083) [A,D]	0.632 (0.100) [A]	0.695 (0.084) [A,B]	158.97	3/158	<0.001
zFe+zTS+zFerritin	- 2.070 (0.246) [B,C,D]	0.612 (0.293) [A,C]	1.887 (0.353) [A,B,D]	0.644 (0.295) [A,C]	37.91	3/158	<0.001

Tf: Transferrin concentration, TS%: transferrin saturation percentage, UIBC: unsaturated iron-binding capacity, zFe+zTS+zFerritin: index of iron overload computed as z Fe + z TS% + z ferritin

Cluster analysis-generated solution: HCC: healthy control children, TDT: transfusion-dependent thalassemia, TDT+LSE: TDT with depression and low self-esteem, TDT+SIRR: TDT with depression and high social-irritability scores.

All results of GLM analysis with age and sex as covariates

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Table 5: Differences in immune biomarkers between healthy control children (HCC), transfusion-dependent-thalassemia (TDT), and TDT with and without depressive symptom domains including lower self-esteem and social-irritability scores.

Variables (z scores)	HCC[A]	TDT[B]	TDT+LSE[C]	TDT+SIRR[D]	F	df	p
Interleukin-1β	-0.825 (0.111) [B,C,D]	0.075 (0.132) [A,C,D]	0.689 (0.159) [A,B]	0.461 (0.133) [A,B]	30.66	3/158	<0.001
Tumor necrosis factor-α	-0.862 (0.109) [B,C,D]	0.237 (0.130) [A,D]	0.287 (0.157) [A]	0.675 (0.132) [A,B]	36.62	3/158	<0.001
CCL-11 (eotaxin)	0. 391 (0.132) [B,C]	-0.228 (0.157) [A]	-0.386 (0.189) [A]	-0.016 (0.158)	5.14	3/158	0.002
Interleukin-10	-0.621 (0.123) [B,C,D]	0.478 (0.147) [A,C]	-0.039 (0.177) [A,B]	0.400 (0.148) [A]	14.11	3/158	<0.001
zTNFα+zIL1β	-1.011 (0.097) [B,C,D]	0.197 (0.115) [A,C,D]	0.571 (0.139) [A,B]	0.680 (0.116) [A,B]	57.31	3/158	<0.001
z(zTNFα+zIL1β)-zIL-10	-0.389 (0.162) [C,D]	-0.282 (0.193) [C,D]	0.609 (0.233) [A,B]	0.280 (0.195) [A,B]	5.96	3/158	0.001
Zinc	-0.285 (0.136)	0.169 (0.161)	-0.012 (0.195)	0.239 (0.163)	2.51	3/158	0.061
Copper	-0.177 (0.138) [B,D]	0.140 (0.164) [A]	-0.075 (0.198)	0.149 (0.166) [B]	1.05	3/158	0.374

Cluster analysis-generated solution: HCC: healthy control children, TDT: transfusion-dependent thalassemia, TDT+LSE: TDT with depression and low self-esteem, TDT+SIRR: TDT with depression and high social-irritability scores.

zTNFα+zIL1β: computed as z interleukin-1β + z tumor necrosis factor-α.

z(zTNFα+zIL1β)-zIL-10: computed as z score of (zTNFα+zIL1β) – z IL-10

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Table 6: Results of multiple regression analysis with symptom domains as dependent variables and iron status and immune biomarkers as explanatory variables.

Dependent Variables	Explanatory Variables	β	t	p	F model	df	p	R^2
#1. Key depression	**Model**				32.30	3/160	<0.001	0.377
	Ferritin	0.401	4.94	<0.001				
	zTNF+zIL-1β	0.247	3.05	0.003				
	Age	0.179	2.86	0.005				
#2. Physiosomatic	**Model**				37.40	3/160	<0.001	0.412
	Ferritin	0.470	6.69	<0.001				
	IL-1β	0.221	3.14	0.002				
	Copper	0.157	2.58	0.011				
#3. Self-esteem	**Model**				50.72	3/160	<0.001	0.487
	Iron	0.280	4.13	<0.001				
	Age	0.440	7.76	<0.001				
	Ferritin	0.334	4.93	<0.001				
#4. SIRR	**Model**				52.81	2/161	<0.001	0.396
	Ferritin	0.439	5.51	<0.001				
	zTNF+zIL-1β	0.250	3.14	0.002				
#5. PC4PC	**Model**				52.92	4/159	<0.001	0.571
	Ferritin	0.443	6.01	<0.001				
	zTNF+zIL-1β	0.252	3.63	<0.001				
	Age	0.134	2.58	0.011				
	TS%	0.165	2.48	0.014				
#6. PC4PCs IN HCC	**Model**				10.72	2/50	<0.001	0.300
	Age	0.444	3.69	0.001				
	Copper	0.249	2.07	0.043				
#7. PC4PCs IN TDT	**Model**				12.84	2/108	<0.001	0.192

	Number transfusions	0.370	4.28	<0.001				
	zTNF+zIL-1 β	0.225	2.60	0.011				

IL-1β: interleukin-1β, TNF-α: tumor necrosis factor-α.

zTNFα+zIL1β: computed as z interleukin-1β + z tumor necrosis factor-α.

SIRR: social-irritability symptoms, PC4PC: first principal component (PC) extracted from four Child Depression Inventory (CDI) subdomains, HCC: healthy control children, TDT: transfusion-dependent thalassemia.

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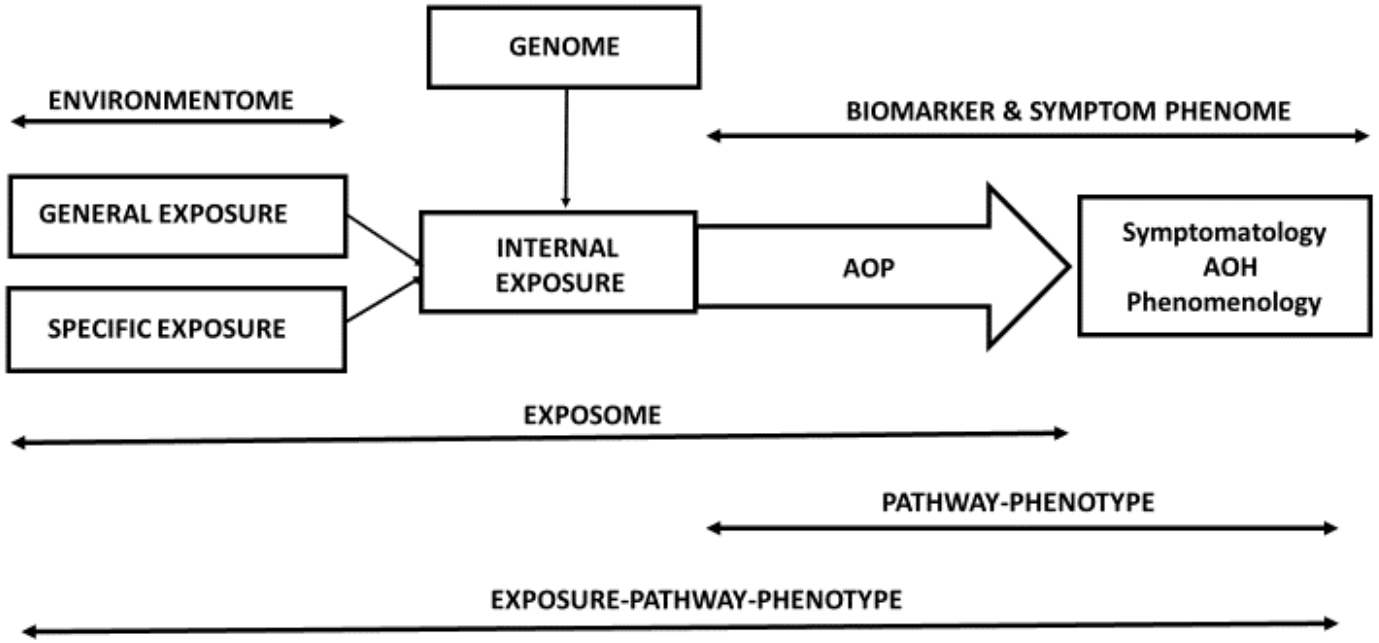

Figure 1: Associations between genome and exposome (consisting of general as well as specific exposures) ultimately leading to adverse outcome pathways (AOP) that cause adverse outcome health effects, namely the clinical phenome of depression (symptoms and phenomenology).

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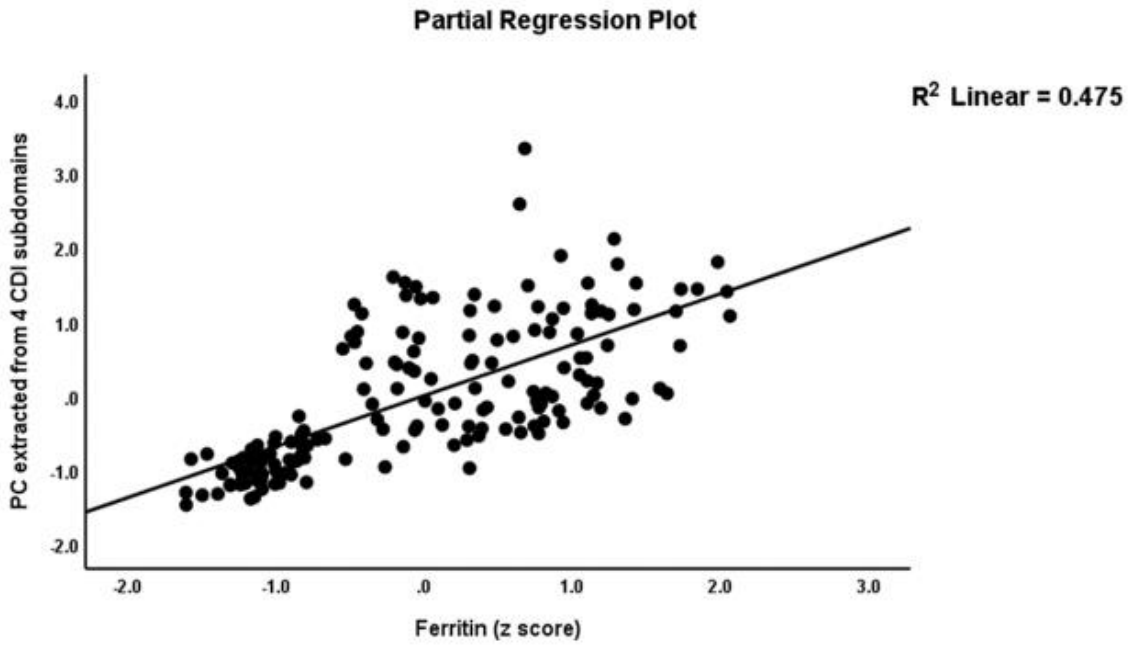

Figure 2: The partial regression of the first principal component (PC) extracted from the four Child Depression Inventory (CDI) subdomains on ferritin after controlling for the effects of sex and age.

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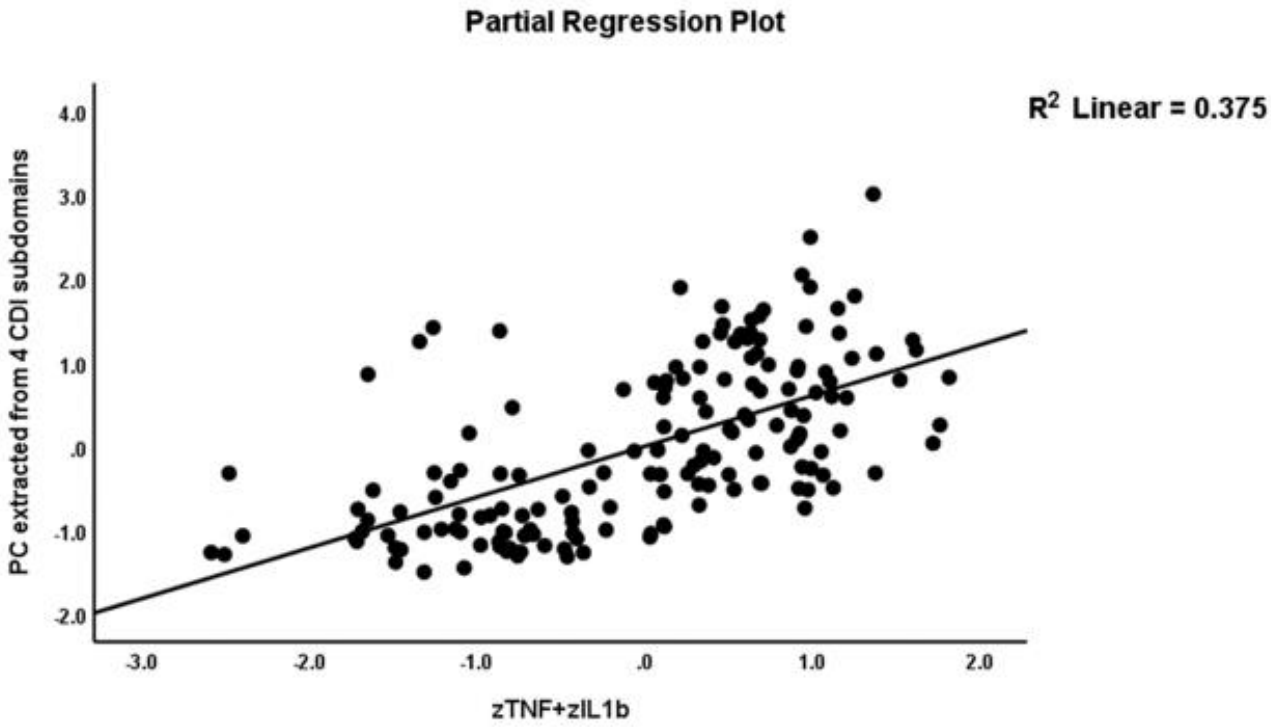

Figure 3: The partial regression of the first principal component (PC) extracted from four Child Depression Inventory (CDI) subdomains on the z unit-weighted composite score zTNF+zIL1 (z scores of tumor necrosis factor-α + z scores of interleukin-1β) after controlling for the effects of sex and age.

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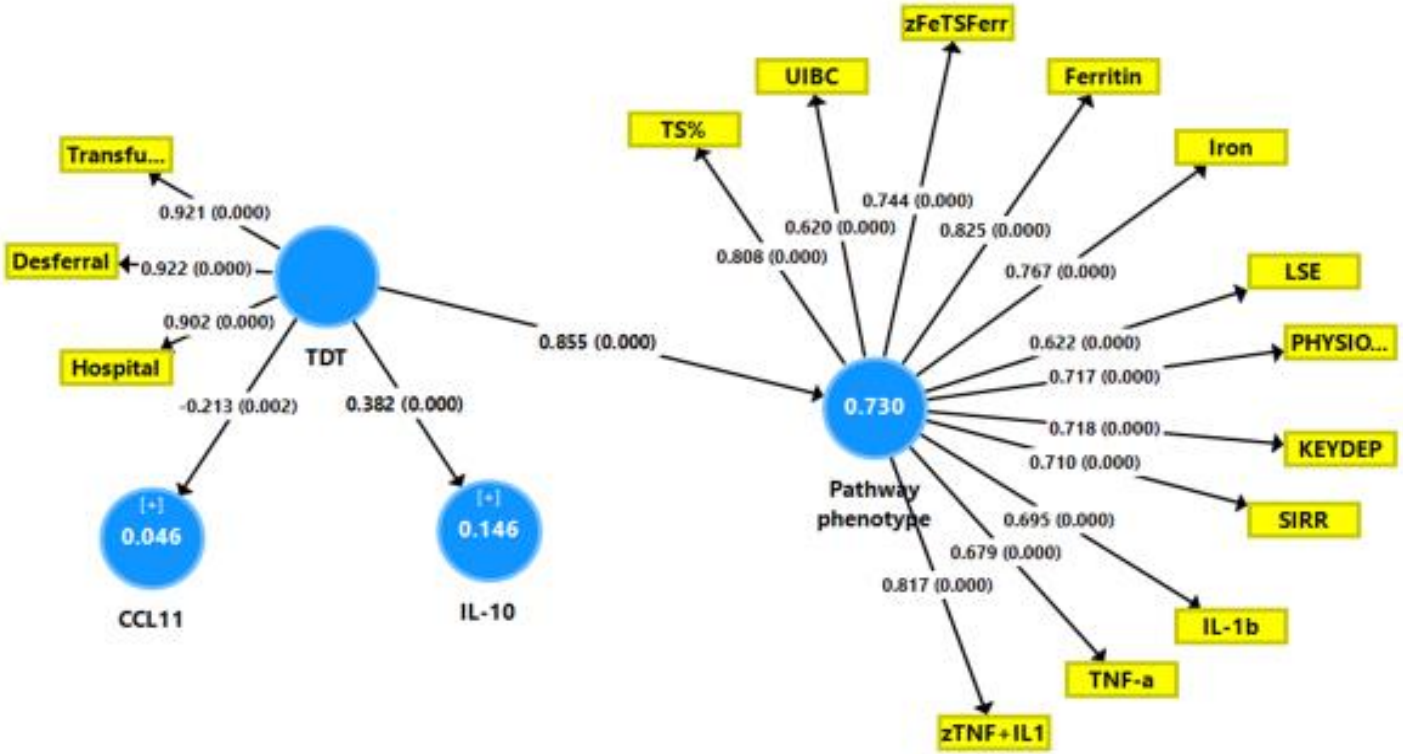

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Figure 4: Results of Partial least Squares (PLS) with construction of a pathway-phenotype based on the four subdomain scores extracted from the Child Depression Inventory, combined with iron overload status and immune biomarkers. Shown are loadings (p values) for the outer model and pathway coefficients (p values) obtained by complete PLS analysis performed on 5000 bootstrap samples.

Transfu: number of transfusions, Hospital: number of hospitalizations, TS%: transferrin saturation percentage, UIBC: Unsaturated Iron binding Capacity, zFeTSFerr: z unit-weighted composite score reflecting iron overload, LSE: lower self-esteem, PHYSIO: physiosomatic symptoms, KEYDEP: core depressive symptoms, SIRR: social-irritability scores, IL-1b: interleukin-1β, TNF-a: tumor necrosis factor-α, zTNF+IL1: z unit-weighted composite score reflecting activated M1 macrophage cells.

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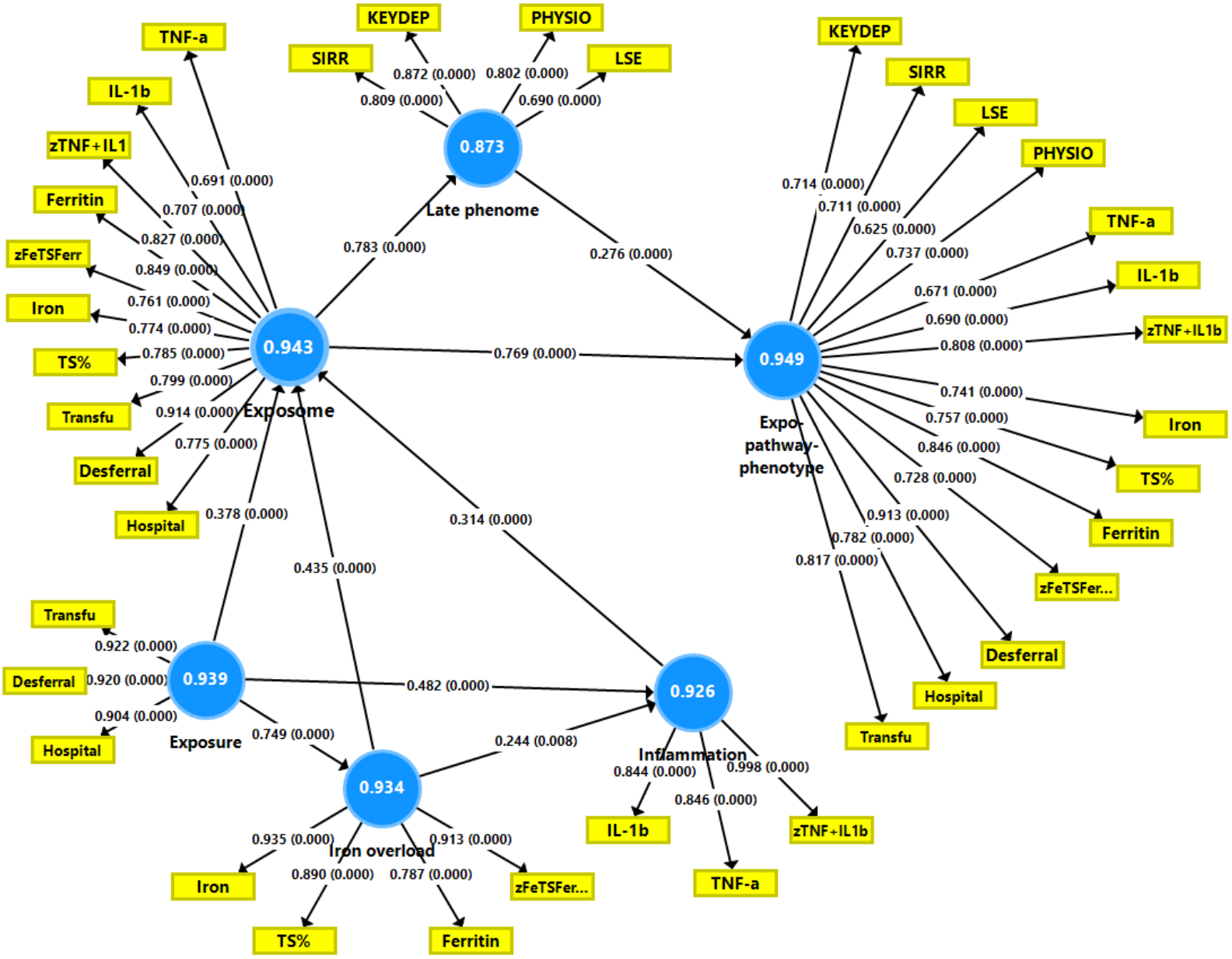

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Figure 5: Construction of higher-order factors, namely an exposure-pathway-phenotype based on latent vectors (LVs) extracted from the symptom phenome (four symptom dimensions) and the exposome. The latter, in turn, is a higher-order construct based on LVs extracted from all inflammatory, iron overload, and external exposure indicators. The latter were extracted from different indicators as shown in this figure. The data in the circles show the composite reliabilities of all constructs

Transfu: number of transfusions, Hospital: number of hospitalizations, TS%: transferrin saturation percentage, zFeTSFerr: z unit-weighted composite score reflecting iron overload, LSE: lower self-esteem, PHYSIO: physiosomatic symptoms, KEYDEP: core depressive symptoms, SIRR: social-irritability scores, IL-1b: interleukin-1β, TNF-a: tumor necrosis factor-α, zTNF+IL1: z unit-weighted composite score reflecting activated M1 macrophage cells.

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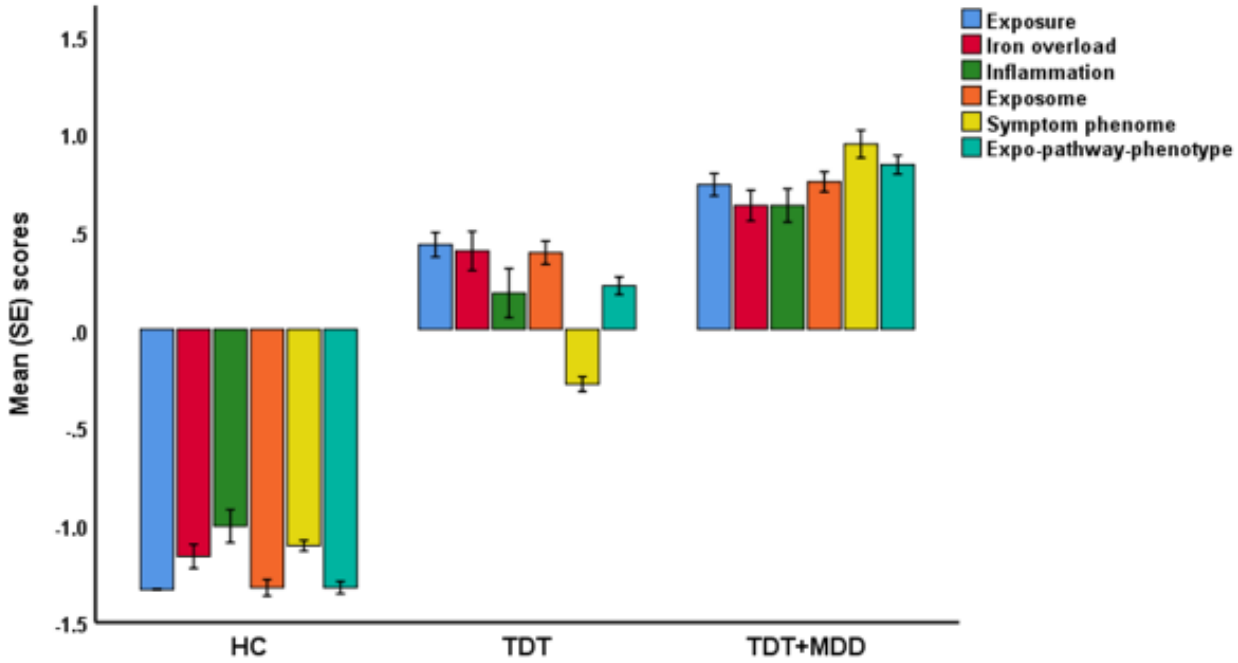

Figure 6: Bar graph (mean z scores ±SE) with latent variable scores in 3 groups, namely healthy children (HC), children with TDT (transfusion-dependent thalassemia) without depression, and TDT+MDD (TDT with depression). The latent scores were computed using the indicators depicted in Figure 5.