Towards a new model and classification of mood disorders based on risk resilience, neuro-affective toxicity, staging, and phenome features using the nomothetic network psychiatry approach.

Short tittle: a nomothetic network model of mood disorders

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Abstract

Current diagnoses of mood disorders are not cross validated. The aim of the current paper is to explain how machine learning techniques can be used to a) construct a model which ensembles risk/resilience (R/R), adverse outcome pathways (AOPs), staging, and the phenome of mood disorders, and b) disclose new classes based on these feature sets. This study was conducted using data of 67 healthy controls and 105 mood disordered patients. The R/R ratio, assessed as a combination of the paraoxonase 1 (PON1) gene, PON1 enzymatic activity, and early life time trauma (ELT), predicted the high-density lipoprotein cholesterol – paraoxonase 1 complex (HDL-PON1), reactive oxygen and nitrogen species (RONS), nitro-oxidative stress toxicity (NOSTOX), staging (number of depression and hypomanic episodes and suicidal attempts), and phenome (the Hamilton Depression and Anxiety scores and the Clinical Global Impression; current suicidal ideation; quality of life and disability measurements) scores. Partial Least Squares pathway analysis showed that 44.2% of the variance in the phenome was explained by ELT, RONS/NOSTOX, and staging scores. Cluster analysis conducted on all those feature sets discovered two distinct patient clusters, namely 69.5% of the patients were allocated to a class with high R/R, RONS/NOSTOX, staging, and phenome scores, and 30.5% to a class with increased staging and phenome scores. This classification cut across the bipolar (BP1/BP2) and major depression disorder classification and was more distinctive than the latter classifications. We constructed a nomothetic network model which reunited all features of mood disorders into a mechanistically transdiagnostic model.

Keywords: mood disorders, major depression, inflammation, neuro-immune, oxidative stress, nitrosative stress, biomarkers

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Introduction

Both major depression disorder (MDD) or unipolar depression and bipolar disorder (BD), type 1 (BP1) and type 2 (BP2), impose considerable disabilities and lowered health-related quality of life (HR-QoL) (Nunes et al., 2018). A Major depressive episode (MDE) is a is a transdiagnostic construct that cuts across BP1, BP2, and MDD and is a more prevalent phenotype than (hypo)mania (Nusslock and Frank, 2011; Corponi et al., 2020). Nevertheless, the case definitions of both MDD and MDE according to DSM (American Psychiatric Association, APA) and ICD (World Health Association, WHO) criteria are rather unreliable (Lieblich et al., 2015). For example, the DSM case definitions of MDD show an intraclass kappa reliability of only 0.28 indicating minimal agreement among psychiatrists (Regier et al., 2013) and, additionally, the reliability of any DSM affective disorder case definition is low (Lieblich et al., 2015). As a consequence, the DSM and ICD taxonomies lack reliability validity and are frequently counterproductive for research purposes (Stoyanov et al., 2020; Hyman, 2010; 2011; Insel et al., 2010).

The ICD and DSM diagnostic classifications are established by professional bodies such as the WHO and APA and are based on consensus criteria using descriptive aspects (Stoyanov, 2020). These ex consensus taxonomies pre-define mood disorder phenotypes before causome and adverse outcome pathways (AOP) are even considered (Stoyanov et al., 2020). As a consequence, the ICD and DSM case definitions of mood disorders are not construed considering state-of-the-art domain knowledge of the causome-AOP constructs, which underpin mood disorders. As such, these case definitions preclude using a deductive or top-down-driven approach. The Research Domain Criteria (RDoC) are more biologically oriented by integrating neurodevelopment, genetic, and environmental factors with cognitive, regulatory, and social processes, and positive and

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negative valence (Insel et al., 2010). However, also the RDoC is driven by expert commitment to define the above-mentioned domains in a top-down concept. Since the current "gold standard" DSM/ICD case definitions of MDD/MDE/BD are unreliable and do not even allow to employ a deductive or top-down-driven approach it is not surprising that decades of psychiatric research did not provide biomarkers or pathways which may be used as external validating criteria.

To overcome these pitfalls, we introduced the nomothetic network psychiatry approach which allows an inductive and bottom-up approach to construct data-driven disease models and evidence-based classifications of mental illness (Maes et al., 2020; Simeonova et al., 2020; Al-Hakeim et al., 2020; Mousa et al., 2020). This method allows to build explicit data models constructed based on causal reasoning using causome (genome and environmentome) feature sets, combined with AOPs, and the clinical phenome, which comprises symptomatome and phenomenome feature sets (Maes et al., 2020). These different feature sets are then integrated and ensembled in unified cause-to-outcome machine learning models, which are consequently cross-validated (Maes et al., 2020). As such, the learned nomothetic network models objectivate the symptomatome and phenomenome of mental illness and translate causome and AOP data into clinical psychiatric scores using computer science, a process called reification (Stoyanov, 2020).

Figure 1 shows a comprehensive mood disorder model based on causal reasoning and previously reported results on the causome, AOPs, and phenome (including staging) in MDD, BP1, and BP2 (Maes et al., 2018; 2019a; 2019b; 2019c; Moreira et al., 2019a; 2019b;Nunes et al., 2018; Moraes et al., 2018; Maes and Carvalho, 2018; Sowa-Kucma et al., 2018a; 2018b; Siwek et al., 2017). Evidence shows that increased reactive oxygen and nitrogen species (RONS) and nitro-oxidative toxicity (NOSTOX) and associated neuro-immune activation are key AOPs in mood disorders (Maes et al., 2010; 2011; Maes et al., 2018; 2019a; 2019b; 2019c). MDD is accompanied

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by increased RONS production, which has caused increased NOSTOX to lipids with consequent aldehyde formation (Maes et al., 2010; 2019a). Elevated protein oxidation with formation of advanced oxidation protein products (AOPP) is a hallmark of both BD1 and MDD, whereas no such aberrations were detected in partially symptomatic remitted BP2 patients (Maes et al., 2019a).

Causome factors including the PON1 Q192R paraoxonase 1 (PON1) gene combined with PON1 paraoxonase enzymatic activity, and early life time trauma (ELT) (environmentome) are associated with later AOPs, the clinical symptomatome and phenomenome of mood disorders (Moreira et al. 2019a; 2019b; Maes et al., 2018; 2019b; Moraes et al., 2018). The lowered PON1 paraoxonase activity, which is associated with the QQ genotype of the Q192R PON1 gene, is strongly associated not only with HDL-cholesterol levels and staging of mood disorders, but also with the symptomatome (increased severity of depression) and the phenomenome (lowered self-rated HR-QoL and increased disabilities) (Moreira et al., 2019a; 2019b). ELTs and especially physical neglect are significantly associated with indices of increased RONS/NOSTOX and sexual abuse is associated with lowered antioxidant defenses (Moraes et al., 2018). Moreover, ELTs including physical and emotional neglect and abuse, as well as sexual abuse are significantly associated with staging features (number of mood episodes and suicidal attempts), increased prevalence of mood disorders, current suicidal ideation, severity of depression and anxiety, and comorbid anxiety disorders (Moraes et al., 2018; Maes et al., 2018; 2019b). The interaction between staging and lowered levels of the PON1-HDL complex significantly mediate the effects of ELT on the symptomatome and phenomenome of mood disorders (Maes et al., 2018).

In the plasma, PON1 is tightly coupled to high-density lipoprotein (HDL) and the complex HDL-PON1 protects against oxidation of HDL, low-density lipoprotein (LDL), and lipid peroxidation and may additionally decrease macrophage oxidative stress by destroying lipid

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peroxides (Moreira et al., 2019a; Mackness and Mackness, 2015). Furthermore, MDD/MDE is characterized by lowered lecithin-cholesterol acyltransferase (LCAT) and glutathione peroxidase activity, and HDL-cholesterol (HDLc) and vitamin E levels, indicating more widespread deficits in lipid-associated antioxidant defenses in mood disorders (Maes et al., 1994; 1997a; 1997b; 2000; 2011; Liu et al., 2015). Importantly, these lowered antioxidant defenses are associated with increased levels of RONS and NOSTOX, indicating that the lowered antioxidant protection contributes to RONS/NOSTOX in mood disorders (Maes et al., 2019a).

Nevertheless, our above-mentioned studies did not construct a nomothetic model by ensembling the causome (ELT and the PON1 genotype), protectome (lowered levels of protective PON1 paraoxonase activity), the AOPs (lowered HDLc-PON1 complex and increased RONS and NOSTOX), and the phenome of mood disorders into a machine-learning derived, explicit data model. Hence, the current publication aims to explain how a) to construct a replicable and reliable nomothetic network of mood disorders using the pre-specified model depicted in Figure 1 as template; and b) to disclose a new classification of mood disorders based on all feature sets that are assembled in the model, namely, causome/protectome, AOPs, staging, symptomatome, and phenomenome scores.

Methods

Subjects

This study recruited 57 heathy volunteers and 115 patients with mood disorders. The latter were admitted as outpatients to the Psychiatric Clinics, University Hospital of the Universidade Estadual de Londrina (UEL), Parana, Brazil. Included were patients and controls of all self-declared ethnicities, both sexes, and aged 18 to 65 years. Exclusion criteria for both patients and

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controls were a) neuro-inflammatory and neurodegenerative disease such as stroke, multiple sclerosis, and Alzheimer's and Parkinson's disease, b) (auto)immune disorders including psoriasis, rheumatoid arthritis, COPD, atherosclerosis, inflammatory bowel disease, systemic lupus erythematosus; hepatitis B and C virus and HIV infection; diabetes type 1; and chronic kidney disease; c) a lifetime history of use of immunomodulatory drugs including glucocorticoids and immunosuppressiva; d) use of therapeutic doses of antioxidant supplements and omega-3 polyunsaturated fatty acids during the past four weeks prior to the study; and e) pregnant and lactating women. We excluded controls with any axis 1 diagnosis and patients with axis 1 diagnosis other than BD and MDD, except anxiety disorders including generalized anxiety disorder, panic disorder, and phobias, and also post-traumatic stress disorder. The healthy volunteers were recruited by word of mouth from the same catchment area as the patients, namely Parana, Brazil. The mood disordered patients were partially symptomatic remitted or symptomatic remitted patients of their index episode, which was not of **(**hypo**)**manic polarity**.** We included 45 BP1, 23 BP2 and 37 MDD patients. All participants provided written informed consent to take part in the study, with the experimental procedures being approved by the Research Ethics Committee at UEL (protocol number: CAAE 34935814.2.0000.5231).

Clinical assessments

Clinical causome assessments

ELT were assessed using the Childhood Trauma Questionnaire (CTQ) in a validated Portuguese translation adapted for use in a Brazilian population (Bernstein et al., 2003; Grassi-Oliviera et al., 2006). The CTQ is a self-rating scale that delineates a history of emotional and

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physical neglect or abuse and sexual abuse during childhood. The frequencies of these 5 types of ELTs are scored on 28 items using a five-point Likert scale.

Phenome and symptomatome assessments

The diagnosis of MDD and BD, and anxiety disorders was made using the Structured Clinical Interview for DSM-IV-TR (clinical version) translated into Portuguese and validated for use in a Brazilian population (Del Ben et al., 2001). The Columbia–Suicide Severity Rating Scale (C-SSRS) was used in a validated Portuguese translation, to score the number of prior suicide attempts and current suicidal ideation (Posner et al., 2011). Severity of illness was assessed using a) the 17-item Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960), the Hamilton Anxiety Rating Scale (HAMA) (Hamilton, 1959), and the Clinical Global Impressions (CGI) scale (Guy, 1976). A semi-structured interview was employed to assess the number of (hypo)manic and depressive episodes, and the medications used, including mood stabilizers, antidepressants, lithium, and antipsychotics. A staging index was computed as explained previously and the patients were divided into three stages, namely a) stage 1: an early stage with few affective episodes and increased current ad lifetime suicidal behaviours; b) stage 2: the relapse-regression stage with increased recurrent episodes and disabilities, lower HR-QoL and impairments in cognitive processing speed; and stage 3, the suicidal-regression stage with increased ELT, a very high recurrence of affective episodes and suicidal behaviors (Maes et al., 2019b).

Phenomenome assessments

We used the WHO Quality of Life Instrument-Abbreviated Version (WHO-QOL-BREF) in a validated Brazilian Portuguese translation to assess HR-QoL (Skevington et al., 2004; Fleck

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et al., 2000). This rating scale assesses four HR-QoL domains, namely physical health, psychological health, social relationships, and environment. The Sheehan Disability Scale was employed to measure disabilities in three domains, namely occupational, social life, and family life (Sheehan et al., 1996).

Sociodemographic and other clinical data

A semi-structured interview was used to assess socio-demographic data such as age, sex, employment, education (in years), and marital status, income, etc. The DSM-IV-TR criteria were employed to make the diagnosis of tobacco use disorder (TUD) and body mass index (BMI) was computed as body mass (kg) divided by square of height (m^2).

Measurements of causome biomarkers

Fasting blood samples were collected at 8:00 am and serum was aliquoted and consequently stored at -80 °C until thawed for assays of the biomarkers. Total PON1 activity, i.e. PON1 Q192R genotype (QQ, QR, and RR) and PON1 paraoxonase enzymatic activity were determined as described previously (Richter et al., 2009; Moreira et al., 2019b). For the stratification of the functional PON1 Q192R genotype we employed 4-(chloromethyl)phenyl acetate (CMPA) (CMPA, Sigma, USA) and phenyl acetate (PA, Sigma, USA) and to determine paraoxonase activity we used CMPA (Moreira et al., 2019a; 2019b). These assays were performed on a spectrophotometer microplate reader (EnSpire, Perkin Elmer, USA). The total study group was at Hardy-Weinberg equilibrium with regard to the PON1 allelic frequencies (χ^2=1.69, df=1, p=0.193).

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Measurements of the AOPs.

We assayed serum levels of HDLc, nitric oxide metabolites (NOx), lipid hydroperoxides (LOOH), malondialdehyde (MDA), AOPP, and red blood cell SOD1 as described previously (Maes et al., 2019a). In brief, HDLc was measured using an automated method with the Dimension RxL (Deerfield, IL, USA). SOD1 activity was assayed by a pyrogallol method. LOOH were measured using a chemiluminescence method (CL-LOOH) conducted in a Glomax luminometer (TD 20/20 Turner Designers, USA). NO metabolites (NOx) was assayed by determining plasma nitrite concentration in a microplate reader Asys Expert Plus, Biochrom (Holliston, MA, USA). MDA was assayed through complexation with two molecules of thiobarbituric acid (TBA) and using high performance liquid chromatography (HPLC Alliance e2695, Waters', Barueri, SP, Brasil). AOPP was assayed employing the method described by Hanasand et al. (2012) in a microplate reader, Perkin Elmer, model EnSpire (Waltham, MA, EUA) at a wavelength of 340 nm. All biomarkers were assayed in a single run by the same operator who was blinded to the clinical diagnosis. The inter-assay coefficients of variability were less than 10% for all biomarkers. As explained previously, we computed two z unit-weighted composite scores, namely z LOOH +z SOD+ z NOx (named RONS leading to lipid peroxidation) and z SOD + z LOOH + z NOx +z MDA +z AOPP (named NOSTOX, namely the pathway from RONS to aldehyde formation and protein oxidation) (Maes et al., 2019a). Moreover, we extracted a LV from both HDLc and PON1 paraoxonase activity reflecting levels of the HDLc-PON1 complex (Maes et al., 2018). Previously, we have found that, using the same study population, there were no significant effects of the drug state on the biomarker results, including use of antidepressants, mood stabilizers, atypical antipsychotics, and lithium (Maes et al, 2019a).

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Statistics

We employed analysis of variance (ANOVA) to check differences in continuous variables between classes, and analysis of contingency tables (χ^2-test) to assess associations between categorical/ordinal variables. To adjust for type 1 errors due to multiple comparisons, we employed a p-correction method, namely the false-discovery rate (FDR) procedure (Benjamini and Hochberg, 1995). Correlations between scale variables were tested using Pearson's product moment and Spearman's rank order correlation coefficients. All tests were two-tailed and an alpha level of 0.05 was considered to be statistically significant. All analyses were performed using the IMB-SPSS software version 25 for Windows.

Partial Least Squares (PLS) pathway analysis (Ringle et al., 2014) was employed to examine the bottom-up, theoretical model displayed in Figure 1. First, we examined whether staging, the symptomatome, and phenomenome data belonged to one and the same LV or to different LVs and, consequently, one or more LVs were used as output variables. An LV extracted from the Q192R genotype (additive model, with QQ=2, QR=1, RR=0) and PON1 paraoxonase 1 activity in a reflective model was used as an input variable (named: PON1genozyme). As such, this LV reflects gene-associated paraoxonase 1 activity. We used two different ELT constructs because previously we showed that physical neglect has different effects on NOSTOX biomarkers as compared with the other ELTs (Moraes et al., 2018). Therefore, physical neglect was entered as a single indicator, whereas the other ELTs were entered as a LV in a reflective model. The HDLc-PON1 complex was entered as a reflective LV extracted from HDLc and PON1 enzymatic activity, and NOSTOX was entered as a single indicator. The PLS path model was considered reliable when the model and constructs complied with pre-specified quality criteria: a) model fit RSMR (standardized root mean residual) < 0.08; b) LV constructs display adequate reliability as

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indicated by rho_A > 0.8 and average variance extracted (AVE) > 0.5; c) all LV indicators show factor loadings > 0.5 at $p<0.001$; and (d) construct cross-validated communalities and redundancies are adequate. Complete PLS path analysis was conducted using 5000 bootstrap samples and path coefficients with t-values and exact p-values were computed as well as total indirect and specific indirect effects and total effects of all input variables. Finally, we computed the latent variable scores reflecting the ELT LV, staging LV, and phenome LV.

Clustering analysis was performed with the aim to classify the subjects into clusters based on causome/protectome, AOP, and phenome scores and to discover a new typology of mood disorders based on all features (bottom-up method). We conducted K-mean, K-median, and Ward's method using SPSS 25 and the Unscrambler (Camo, Oslo, Norway). To interpret the cluster analysis-generated classes, we a) conducted analysis of contingency tables (X^2-test) and ANOVAS; and b) displayed the key feature sets (in z values) of the new classes in clustered bar graphs.

Results

Construction of a Risk Resilience index

Using the causome/protectome variables, we computed a z unit-weighted composite score, which reflects the risk/resilience (R/R) ratio, namely z (LV extracted from physical and sexual abuse, emotional neglect and abuse) + z physical neglect – z (LV extracted from PON1 Q192R genotype in an additive model and PON1 paraoxonase activity). Using a visual binning method these R/R values were then binned into three groups with a comparable number of subjects. **Table 1** shows the R/R measurements used to form these groups, namely low R/R (low ELT, high PON1 enzymatic values), moderate R/R group (high ELTs, high QQ prevalence, low PON1 activity), and

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high R/R group (very high ratings on all ELTs, high QQ prevalence, low PON activity). There were no significant differences in age, sex, BMI, education, and TUD between these three R/R groups. The employment rate was significantly lower in the high R/R group than in the low R/R group.

Higher Risk/Resilience predicts the AOPs

The R/R ratio was significantly correlated with HDLc (r=-0.170, p=0.026, all n=172), HDLc-PON1 complex (r=-0.431, p<0.001), RONS (r=-0.236, p=0.002), and NOSTOX (r=-0.179, p=0.019). **Table 2** shows the measurements of the AOP variables in these three RR groups indicating that HDLc was significantly lower and RONS and NOSTOX significantly higher in the moderate and high R/R groups as compared with the low R/R group. The HDLc-PON1 complex values were significantly lower in the high R/R group than in the low R/R group. These four group differences remained significant after p-correction for FDR.

Higher Risk/Resilience predicts staging

The R/R ratio was significantly correlated with the number of depressive episodes (r=0.431, p<0.001)), number of (hypo)mania episodes (r=0.300, p<0.001), number of all episodes (r=0.413, p<0.001), number of suicidal attempts (r=0.403, p<0.001). **Table 2** shows that the number of depressive and (hypo)mania episodes, and the total number of episodes and suicidal attempts was significantly higher in the high R/R group than in the two other groups. There was a significant association between the R/R groups and the staging groups as defined earlier with increased stage 2 in the moderate R/R group, and increased stage 3 in the high R/R group. These intergroup differences remained significant after FDR p-correction.

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Lower Risk Resilience predicts the symptomatome

Table 3 shows the significant association between the R/R groups and the clinical diagnosis with an increased frequency of mood disorders in the moderate and high R/R groups. There was no significant association between the R/R groups and the BP1/BP2/MDD groups. We found that the frequency of current suicidal ideation was significantly higher in the high R/R group than in the moderate and low R/R groups. We found significant correlations between the R/R score and the HAM-D ($r=0.403$, $p<0.001$), HAM-A ($r=0.409$, $p<0.001$), and CGI ($r=0.416$, $p<0.001$) scores. Table 3 shows that the HAM-D, HAM-A, and CGI scores were significantly higher in the moderate and high R/R groups as compared with the low R/R group.

Lower Risk Resilience predicts the phenomenome (lowered HR-QoL)

We found significant correlations between the R/R score and Sheehan 1 ($r=0.381$, $p<0.001$), Sheehan 2 ($r=0.467$, $p<0.001$), Sheehan 3 ($r=0.338$, $p<0.001$), WHO-QoL domain 1 ($r=-0.422$, $p<0.001$), domain 2 ($r=-0.481$, $p<0.001$), domain 3 ($r=-0.482$, $p<0.001$), and domain 4 ($r=-0.366$, $p<0.001$). Table 3 shows the WHO-QoL domain 1, 2, and 3 score were significantly lower in the high R/R group than in the two other R/R groups. The WHO-QoL domain 3 scores were significantly different between the three R/R groups and decreased from the low R/R to the moderate R/R to the high R/R group. These interclass differences remained significant after FDR p-correction.

Construction of a PLS pathway model.

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Figure 2 shows the final PLS pathway model with a phenome LV (extracted from symptomatome and phenomenome data) as final outcome variable. Staging and the phenome LV could not be combined because the loading on the staging variables were < 0.5. The input variables were PON1genozyme, ELT LV, and physical neglect, predicting the HDLc-PON1 complex, NOSTOX, and all those input variables predicting staging and, consequently, the phenome LV. Sex, age, BMI, and TUD were entered as additional single indicators allowing to predict all other variables. This final model only shows the significant paths. The SRMR overall fit of this PLS pathway model (0.049) and the construct reliabilities of the phenome LV (rho_A=0.932 and AVE=0.586), ELT LV (rho_A=0.869, AVE=0.658), staging LV (rho_A=0.870, AVE=0.678), and PON1genozyme (rho_A=0.879, AVE=0.736) LVs were more than adequate. The outer model loadings on these 4 LVs were > 0.5 at $p<0.001$ and the construct cross-validated communalities and redundancies were accurate. We found that 44.2% of the variance in the phenome LV could be explained by the regression on staging, ELT and NOSTOX (positively), and HDLc-PON1 complex (inversely). We found that 20.4% of the variance in the staging LV was explained by PON1genozyme and ELT, 53.8% of the variance in the HDLc-PON1 complex by PON1genozyme (positively), BMI, and TUD (both inversely). Finally, 27.0% of the variance in NOSTOX was explained by HDL-PON1 complex (inversely) and TUD, age, male sex, and physical neglect (all positively).

There were significant specific indirect effects of PONgenozyme on the phenome LV mediated by a) HDLc-PON1 complex (t=2.11, p=0.035), and b) staging (t=2.50, p=0.012). There was a significant specific indirect effect of ELT on the phenome mediated by staging (t=4.72, $p<0.001$). There were significant total effects of ELT (t=10.27, $p<0.001$), PON1genozyme (t=11.16, $p<0.001$), HDL-PON1 complex (t=2.95, p=0.005), NOSTOX (t=2.27, p=0.023), and

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staging (t=5.75, p<0.001) on the phenome. In addition, there were also total effects of TUD (t=2.91, p=0.004) and BMI (t=2.04, p=0.041), but not age (t=0.41, p=0.158) and sex (t=1.71, p=0.055) on the phenome.

A new bottom-up classification of mood disorders

Consequently, we have calculated latent variable scores (LS) based on the staging and phenome LVs and computed an overall AOP reflecting neuro-affective toxicity as z NOSTOX – z HDL-PON1 complex (NOSTOX/ANTIOX). Using these three composite scores and the R/R score we conducted K means, K-median, and Ward's clustering methods which yielded similar results with a three-class solution. Figure 3 shows the z values of the R/R, NOSTOX/ANTIOX, staging, and phenome scores. All controls were allocated to a first cluster, whereas patients were divided into two clusters a first (cluster 1) with 32 patients, and a second (cluster 2) with 73 patients. Moreover, we re-conducted the cluster analysis with the diagnoses of MDD, BD, BP1 and/or, BP2 as additional variables, but this had no effect on the outcome of the clustering analysis. ANOVAs showed that the R/R (F=27.44, df=1/103, p<0.001), NOSTOX/ANTIOX (F=80.91, df=1/103, p<0.001), and staging (F=4.78, df=1/103, p=0.031) scores were significantly higher in cluster 2 than in cluster 1, whereas there were no significant differences in the phenome LS (F=2.92, df=1/103, p=0.090).

Table 4 shows that there were no significant differences in age, sex, BMI, and TUD between these 3 groups. Education was significantly lower in cluster 2 than in controls and the unemployment rate was significantly higher in patients than in controls, but not different between the two patient clusters. There were no significant differences in the distribution of BP1 and MDD among the clusters, whereas BP2 was higher in cluster 1 than 2. There were more patients with

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stages 2 and 3 in cluster 2 than in cluster 2. There were no differences in the prevalence of anxiety disorders and post-traumatic stress disorder between both patient clusters (results not shown).

Discussion

The bottom-up approach using R/R as explanatory variable

The first major finding of this study is that a newly constructed risk/resilience (R/R) index significantly predicted all downstream feature sets of mood disorders, namely AOPs, staging, and the phenome scores. The variables employed to compute this R/R index were pre-processed and re-engineered as a z unit-weighted composite score, which reflects the ratio between increased ELT and the QQ genotype (two risk factors) and PON1 paraoxonase activity (a protective factor). Increased values on this R/R index therefore indicate increased risk and lowered resilience and, accordingly, three categories were formed reflecting low, high, and very high R/R values. These R/R categories were then used as the explanatory variables in regression analysis or GLM analyses predicting the downstream AOP, staging, and phenome scores, which were entered as dependent variables. This approach contrasts with the common biological psychiatry approach, which uses non-validated taxonomies (DSM and ICD) in top-down experiments whereby predefined mood disorder classes (e.g. versus controls) are entered as explanatory variables in GLM analyses or ANOVAs to detect changes in causome (e.g. ELTs), AOP, and other phenome data. Nevertheless, causal reasoning indicates that the R/R index predicts the downstream feature sets (Maes et al., 2020) and, thus, should be used as the explanatory variable. By inference the use of diagnostic classes as explanatory variables confuses the consequence with the causes and, therefore, is grossly inaccurate (Maes et al., 2020). Phrased differently, most biological psychiatry research not only uses unreliable diagnostic classes but also inappropriate statistical analyses with inadequate

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outcome measures and model assumptions, which have invalidated the results. Moreover, the DSM and ICD case definitions of mood disorders are not validated (Maes et al., 2020; Stoyanov, 2020) and the psychiatric reasoning used to make the diagnoses is based on the patients' narratives and is prone to cognitive bias by the subjective illness scripts of the psychiatrist raters. Therefore, it is not surprising that decades of causome and AOP research did not provide evidence-based tools to externally validate this kind of taxonomies.

A new nomothetic model of mood disorders

The second major finding of this study is that we were able to construct a reliable nomothetic model that ensembles the R/R, AOPs, and phenome data in a new data-driven, bottom-up, cause-to-phenome model of mood disorders. Our novel nomothetic model reflects how changes in the causome and/or protectome (resilience) may lead to AOPs reflecting lowered lipid-associated antioxidant levels and increased NOSTOX, which in turn may affect staging and the phenome of mood disorders. It is important to note, that a large part of the phenome of mood disorders (44.2%) could be explained by the cumulative effects of ELTs, AOPs, and staging, and that the latter mediates in part the effects of PON1 gene and ELTs on the phenome.

Previously, we constructed another nomothetic model of mood disorders unifying other causome (IgA/IgM responses to lipopolysaccharides, LPS), AOP (total peroxides; IgG to oxidized low density lipoprotein or LDL; and IgM to oxidative specific epitopes and NO-adducts or OSENO); and phenome (clinical ratings of severity of illness, treatment resistance, and polarity) feature scores (Simeonova et al., 2020). This model displayed that increased LPS load in the plasma (a possible causome factor) may lead to elevated RONS and NOSTOX, which, in turn, may lead to the phenome (Simeonova et al., 2020). By inference, in both models (the current study and Simeonova et al. 2020) RONS/NOSTOX biomarkers are key AOPs, which may cause a

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multitude of detrimental effects. As explained previously (Maes and Carvalho, 2018; Maes et al., 2011; Moylan et al., 2014; Anderson and Maes, 2015), RONS (increased total peroxides, LOOH, NOx, and SOD1), and elevated lipid peroxidation and aldehyde formation (increased MDA), protein oxidation (increase AOPP), and increased (auto)immune responses to OSENO (IgG to oxidized LDL and IgM to OSENO) may cause neurotoxic effects via a multitude of mechanisms leading to the features of mood disorders, a phenomenon which we proposed to name "neuro-affective toxicity" (Maes et al., 2020; Simeonova et al., 2020).

As such, our bottom-up, nomothetic model approach presents a comprehensive model of mood disorders as compared with the top-down approach advocated by APA, WHO, and RDoc criteria. In addition, our models objectivate the symptomatome and the phenomenome of mood disorders because the model "translates" the impact of R/R and AOPs to clinical scores, and vice versa (Stoyanov, 2020). Phrased differently, the art of identifying complex psychiatric disorders should not rely on clinical reasoning using unreliable top-down case definitions, but on a data-driven modelling approach using machine learning to construct reliable and replicable nomothetic network models, which unify the building blocks of mental illness, namely causome, AOP, and phenome scores. It is interesting to note that our nomothetic network models treat the descriptive and narrative clinical concepts as well phenomenome features as material concepts, a phenomenon named reification of diagnosis (Stoyanov, 2020).

Distinctive clusters of patients

The third major finding of this study is that, using clustering analysis performed on the R/R, AOP, staging, and phenome scores, we discovered two distinctive clusters of patients. The first cluster comprised 69.5% of the patients and was characterized by increased R/R, AOP, and staging as compared with the other cluster, whilst the latter was separated from controls by

increased staging and phenome scores only. Likewise, a previous study (Simeonova et al., 2020) found that clustering analysis performed on causome, AOP (NOSTOX/antioxidant ratio), and phenome data, allocated 74.3% of the patients with mood disorders to an AOP-driven cluster, indicating RONS/NOSTOX disorders in the majority of mood disordered patients.

In the current study, we observed that more MDD/BP1 patients were allocated to the cluster with higher R/R and AOP scores, while more BP2 patients were allocated to the cluster without such disorders. Also, Simeonova et al. (2020) found that a large part of MDD/BP1 patients (84.2%) were allocated to a cluster with activated RONS/NOSTOX pathways, whereas a considerably lower percentage of patients with BP2 (44%) were allocated to the same class. Such findings agree with previous studies indicating that MDD/BP1 and BP2 may be discriminated using biomarkers. Thus, increased AOPP concentrations were established in MDD and BP1 versus BP2 and healthy volunteers (Maes et al., 2019a) and higher MDA levels were detected in MDD versus BP2, with BP2 patients occupying an intermediate position (Maes et al., 2019a). Not all reports, however, observed significant differences in lipid peroxidation between MDE patients with BP1, BP2, or MDD (Sowa-Kucma et al., 2019b).

Last, but not least, both the current study and the study by Simeonova et al. (2020) report that the cluster analytically-generated classification based on causome, AOP, and phenome scores straddled across the MDD/BP1/BP2 taxonomies and, importantly, was by far more influential than the MDD/BP1/BP2 classification. A such, both the current study and the study from Simeonova et al. (2020) constructed a mechanistically transdiagnostic model and patient cluster which was characterized by neuro-affective toxicity.

Because the classification into MDD, BP1, and BP2 is less distinctive than the cluster analysis-derived solution, it is probably more adequate to use the latter clusters to define mood

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disorders subgroups rather than the MDD/BP1/BP2 taxonomies. Accordingly, these new clusters of patients may be given a new name, which should describe the key AOP and phenome feature sets. The name proposed by Simeonova et al. (2020) to describe their new RONS/NOSTOX cluster seems appropriate, namely "Major DysMood Disorder due to neuro-affective toxicity" (MDMD-NAT). Cluster 1, on the other hand, could be named "DysMood Disorder" (DMM) to describe moderately increased staging and phenome scores. Future research should examine the causome and AOPs in the latter subgroup by adding the above-mentioned biomarkers.

Limitations

Some limitations should be considered when interpreting the results. Firstly, in a case-control study, deductions on casual relationships should be made carefully. Nevertheless, causal reasoning indicates that the pathways from the R/R ratio to the AOPs and phenome can be validated because it comprises a genotype with associated enzyme activity and ELTs which both increase vulnerability to AOPs and the phenome of mood disorders (Moraes et al., 2018; Maes et al., 2019; 2020). Secondly, future research should conduct predictive modeling on a larger number of patients and cross-validate optimized cluster classifications. Thirdly, the model should be enriched by the inclusion of more causome, AOP, and phenome data: a) general environmentome factors including nutrition and environmental toxins, and lifestyle behaviors; b) other well-known biomarkers of mood disorders, including acute phase proteins, cytokines, CD markers, LPS load in plasma, and autoimmune responses to OSENO (Maes and Carvalho, 2018; Simeonova et al., 2020); and c) in vivo histology magnetic resonance imaging and spectroscopy in order to pinpoint the functional "brainome" factors that are caused by neuro-affective toxicity.

Conclusions

In conclusion, in this paper we explained how to construct a nomothetic network model that ensembles causome, AOPs, and phenome features in a machine learning model. Based on this data-driven modelling of feature sets, cluster analysis disclosed two new diagnostic clusters of patients which should be named “MDMD-NAT” and “DMM”. We found that 69.5% of the mood disorder patients and 74.4% of the BP1/MDD patients were allocated to the MDMD-NAT cluster. This shows that a meaningful part of patients with MDD/BP1/BP2 should be treated transdiagnostically targeting antioxidant levels, RONS and NOSTOX.

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Authorships

All authors contributed to the writing up of the paper. The work was designed by SOVN, MM, DSB, JBM and HOV. Data were collected by SOVN, HOV, AC, and JBM. Laboratory analyses

were conducted by KLB, APM and DSB. Statistics were performed by MM. All authors revised and approved the final draft.

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Table 1. Socio-demographic data of the participants in this study divided according to the risk / resilience (RR) index

Variables	**Low R/R [A] n=57**	**Moderate R/R [B] n=58**	**High R/R [C] n=57**	**F/χ2**	**df**	**p**
PON1 Q192R QQ/QR/RR	8/27/13	30/18/4	29/16/5	0.682	-	<0.001
PON1 CMPAase (U/mL)	36.89 (9.40) [B,C]	22.79 (7.41) [A]	22.66 (7.43) [A]	49.88	2/147	<0.001
Sex abuse (No/Yes)	5.5 (1.4) [C]	5.6 (1.8) [C]	8.3 (5.2) [A,B]	13.65	2/169	<0.001
Physical abuse (No/Yes)	6.6 (2.0) [C]	6.8 (2.7) [C]	12.4 (4.7) [A,B]	55.68	2/169	<0.001
Emotional abuse (No/Yes)	6.9 (2.8) [B,C]	8.7 (3.6) [A,C]	15.6 (5.3) [A,B]	73.72	2/169	<0.001
Emotional neglect (No/Yes)	7.4 (3.1) [B,C]	9.4 (4.5) [A,C]	16.8 (4.6) [A,B]	81.92	2/169	<0.001
Physical neglect (No/Yes)	5.8 (1.4) [B,C]	6.9 (2.1) [A,C]	11.9 (3.4) [A,B]	98.18	2/169	<0.001
Age (years)	4.0 (12.2)	43.7 (10.9)	42.7 (10.2)	0.31	2/169	0.731
Sex (F/M)	39/18	41/17	49/8	5.55	2	0.066
BMI (kg/m2)	25.9 (4.7)	25.9 (4.6)	27.8 (5.0)	2.86	2/161	0.060
MetS (No/Yes)	39/18	36/22	32/25	1.83	2	0.401
Education (years)	11.8 (5.7)	11.7 (5.4)	9.9 (4.2)	2.42	2/168	0.092
TUD (No/Yes)	29/28	27/31	21/36	2.38	2	0.303
Employed (No/Yes)	18/38 [C]	23/35	32/25 [A]	6.98	2	0.031

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Results are shown as mean (±SD). [A,B,C] post-hoc differences between the three categories. All results of analysis of variance (F), analysis of contingency tables ($\chi 2$).

BMI: body mass index; MetS: metabolic syndrome; TUD: tobacco use disorder. PON1 Q192R QQ/QR/RR: paraoxonase 1 genotypes; CMPAase: PON1 CMPAase 4-(chloromethyl phenyl acetate-ase activity.

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Table 2. Measurements of the adverse outcome pathways (AOPs) and staging features in participants divided according to the risk / resilience (R/R) index

Variables	**Low R/R** [A] **n=57**	**Moderate R/R** [B] **n=58**	**High R/R** [C] **n=57**	**F**	**df**	**p**
HDLc*	0.169 (0.121) [B,C]	-0.297 (0.118) [A]	-0.248 (0.128) [A]	4.56	2/156	0.012
HDLc–PON1 complex*	0.750 (0.142) [C]	-0.408 (0.138)	-0.470 (0.150) [A]	23.51	2/156	<0.001
RONS*	-0.610 (0.263) [B,C]	0.048 (0.257) [A]	0.444 (0.279) [A]	3.92	2/156	0.022
NOSTOX*	-0.659 (0.255) [B,C]	0.388 (0.248) [A]	0.363 (0.270) [A]	5.55	2/156	0.005
# depressions	1.7 (3.6) [C]	2.8 (4.0) [C]	4.9 (4.9) [A,B]	8.32	2/166	0.001
# (hypo)mania	1.3 (2.8) [C]	2.2 (3.9) [C]	4.6 (7.5) [A,B]	6.22	2/169	0.002
# all episodes	2.9 (5.4) [C]	5.0 (7.1) [C]	9.6 (10.7) [A,B]	10.07	2/166	0.001
# suicidal attempts	0.5 (3.0) [C]	0.7 (1.6) [C]	2.2 (3.7) [A,B]	5.73	2/169	0.004
Staging HC/1/2/3	34/17/5/1 [B,C]	21/16/18/3 [A,C]	12/2/12/31 [A,B]	78.75	6	<0.001

Results are shown as mean (±SD). [A,B,C] post-hoc differences between the three categories. *Results are shown as mean (±SEM) in z scores after covarying for age, sex, BMI, metabolic syndrome, and tobacco use disorder.

[A,B,C] post-hoc differences between the three categories. All results of analysis of variance.

HDLc: high density lipoprotein cholesterol; HDLc-CMPAase: latent vector extracted from both HDLc and paraoxonase 1 chloromethyl phenyl acetate-ase activity; RONS: index of reactive oxygen and nitrogen species; NOSTOX: index of nitro-oxidative stress toxicity; #: number of ..

Table 3. Measurements of the symptomatome and phenomenome in participants divided according to a risk resilience (RR) index

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Variables	**Low R/R [A] n=57**	**Moderate R/R [B] n=58**	**High R/R [C] n=57**	**F/χ2**	**Df**	**p**
HC/MOOD	34/23 [B,C]	21/37 [A]	12/45 [A]	18.13	2	<0.001
BP1/BP2/MDD	8/5/10	14/9/14	23/9/13	2.49	2	0.647
Current suicidal ideation (No/Yes)	35/18 [C]	29/27 [C]	16/41 [A,B]	16.29	2	<0.001
HAM-D	4.3 (5.7) [B,C]	8.1 (6.8) [A]	9.1 (6.3) [A]	9.13	2/169	<0.001
HAM-A	7.6 (7.2) [B,C]	13.5 (11.1) [A]	14.9 (9.9) [A]	9.691	2/158	<0.001
CGI	2.4 (1.3) [B,C]	3.4 (1.7) [A]	3.8 (1.2) [A]	14.25	2/165	<0.001
Sheehan 1	2.2 (3.2) [B,C]	3.5 (3.6) [A,C]	4.9 (3.6) [A,B]	9.2	2/169	<0.001
Sheehan 2	1.7 (2.6) [B,C]	3.8 (3.7) [A,C]	5.6 (3.7) [A,B]	19.15	2/168	<0.001
Sheehan 3	1.9 (3.2) [B,C]	3.5 (3.5) [A, C]	5.0 (3.9) [A,B]	11.01	2/168	<0.001
WHO Qol Domain 1	27.4 (4.6) [C]	25.7 (6.1) [C]	22.4 (5.9) [A,B]	12.05	2/169	<0.001
WHO Qol Domain 2	22.5 (3.7) [C]	20.8 (5.5) [C]	17.4 (4.8) [A,B]	16.27	2/167	<0.001
WHO Qol Domain 3	11.4 (2.6) [B,C]	10.1 (2.6) [A,C]	8.4 (2.7) [A,B]	18.00	2/169	<0.001
WHO Qol Domain 4	29.2 (4.4) [C]	28.8 (5.9) [C]	25.2 (5.1) [A,B]	10.54	2/169	<0.001

Results of symptoms are shown as mean (±SEM) after covarying for age and sex, or as mean (SD).

[A,B,C] post-hoc differences between the three categories. All results of analysis of variance.

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HC/1/2/3: healthy controls and patients divided into three staging groups; HC/BP1/BP2/MDD: healthy controls, bipolar disorder type 1/ type 2/ major depression; HC/MOOD: healthy controls / mood disorders (that is BP1+BP2+MDD); HAM-D and HAM-A: Hamilton Depression and Anxiety rating Scale; CGI: Clinical Global Impression.

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Table 4. Socio-demographic and clinical data of the healthy controls and patients divided by means of cluster analysis.

Variables	**HC** [A] **n=67**	**Cluster 1** [B] **n=32**	**Cluster 2** [C] **n=73**	**F/χ2**	**df**	**p**
Age (years)	43.1 (11.62)	44.7 (11.4)	41.8 (10.4)	0.78	2/169	0.458
Sex (F/M)	44/23	25/7	60/13	5.29	2	0.071
BMI (kg/m2)	26.3 (4.7)	25.2 (4.3)	27.2 (5.2)	1.95	2/162	0.146
MetS (No/Yes)	41/26	19/13	47/26	0.29	2	0.867
Education (years)	12.6 (5.6) [C]	10.6 (5.0)	10.1 (4.6) [A]	4.48	2/168	0.013
TUD (No/Yes)	32/35	11/21	34/39	1.74	2	0.430
Employed (No/Yes)	14/52 [B,C]	18/14 [A]	41/32 [A]	20.27	2	<0.001
BP1	-	22/10	38/35	2.53	1	0.112
BP2	-	21/11 C	61/12 [B]	4.18	1	0.041
MDD	-	21/11	47/26	0.02	1	0.902
Staging (1/2/3)	-	15/12/5 [C]	20/23/30 [B]	7.10	2	0.029

Results are shown as mean (±SD). [A,B,C] post-hoc differences between the three categories. All results of analysis of variance (F) or analysis of contingency tables (χ2).

BMI: body mass index; MetS: metabolic syndrome; TUD: tobacco use disorder. BP1/BP2: bipolar disorder type 1 and type 2, respectively. MDD: major depressive disorder.

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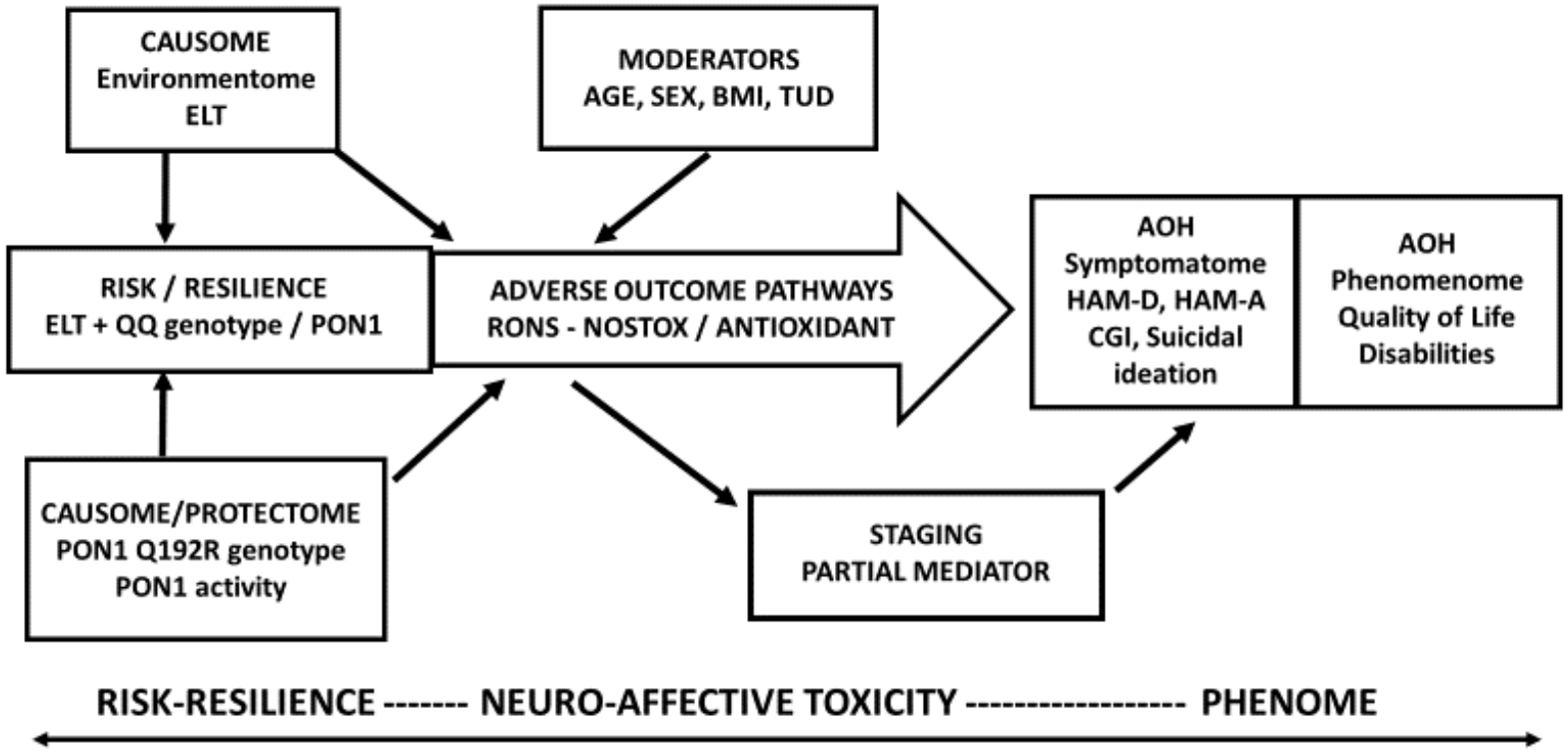

Figure 1. A comprehensive mood disorder model based on causal reasoning and previously reported results on the causome, adverse outcome pathways (AOPs), and phenome features of mood disorders.

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ELT: early lifetime trauma; PON1: paraoxonase 1 Q192R genotype; RONS: reactive oxygen and nitrogen species; NOSTOX: nitro-oxidative stress toxicity; BMI: body mass index; TUD: tobacco use disorder; AOH: adverse health outcomes; HAM-D: severity of depression; HAM-A: severity of anxiety; CGI: clinical global impression

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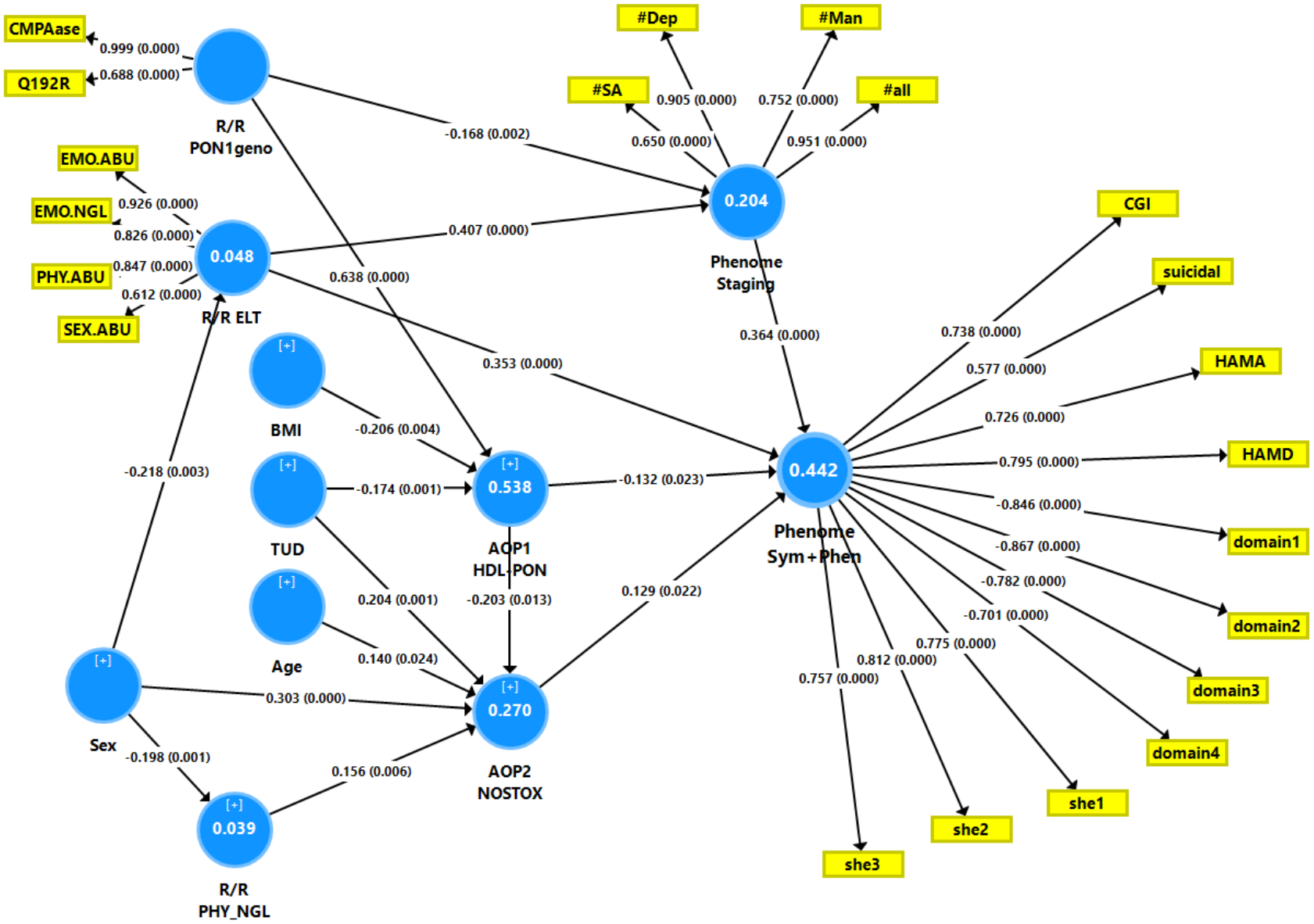

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Figure 2. Results of Partial least Squares (PLS) pathway Analysis. Examined are the effects of risk/resilience (R/R) on adverse outcome pathways (AOPs) and phenome features including staging and symptomatome and phenomenome (Sym+Phen).

The R/R comprises paraoxonase (PON1) genotype and PON1 activity; early lifetime trauma (ELT) such as emotional abuse (EMO.ABU), emotional neglect (EMO.NGL), physical abuse (PHY.ABU), and sexual abuse (SEX.ABU); and physical neglect (PHY.NGL). AOP1: a combination of high-density lipoprotein cholesterol (HDL) and PON1 activity, reflecting antioxidant activity; AOP2: a z unit-weighted composite score reflecting nitro-oxidative stress toxicity. Staging is a latent vector extracted from staging variables, namely number of depressive (#Dep), (hypo)mania (#Man), all (#all) episodes, and suicide attempts (#SA). The symptomatome and phenomenome comprises: current suicidal ideation, clinical global impression (CGI), Hamilton Depression and Anxiety Rating Scale (HAMD and HAMA) scores, domains 1-4 of the WHO-Quality of Life questionnaire, and domains 1-3 of the Sheehan Disability Scale (she1-3).

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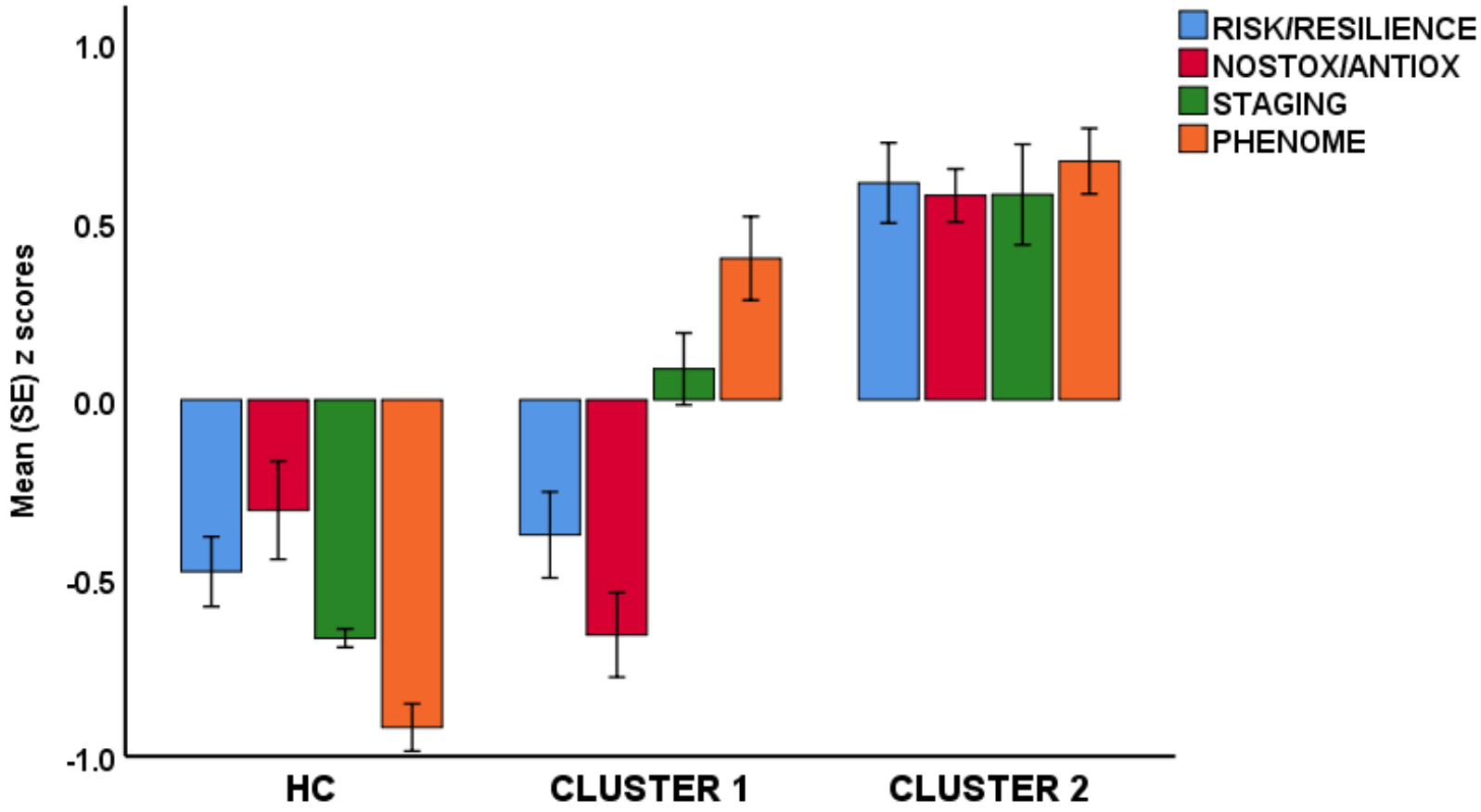

Figure 3. Clustered bar graph showing the results of cluster analysis yielding three groups, healthy controls (HC) and mood disorder patients divided into two clusters.

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Risk/Resilience: a score based on measurements of paraoxonase (PON1) genotype and PON1 activity; early lifetime trauma (ELT) such as emotional abuse (EMO.ABU), emotional neglect (EMO.NGL), physical abuse (PHY.ABU), and sexual abuse (SEX.ABU); and physical neglect (PHY.NGL). NOSTOX/ANTIOX: reflecting the ratio of nitro-oxidative stress toxicity on antioxidant defenses. Staging: variable scores extracted from staging data, namely number of depressive, (hypo)mania and all episodes, and suicide attempts. Phenome: latent variable scores extracted from suicidal ideation, clinical global impression score, the Hamilton Depression and Anxiety Rating Scale scores, domains 1-4 of the WHO-Quality of Life questionnaire, and domains 1-3 of the Sheehan Disability Scale.