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In schizophrenia, psychomotor retardation is associated with executive and memory impairments, negative and psychotic symptoms, neurotoxic immune products and lower natural IgM to malondialdehyde.

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Abstract

BACKGROUND: Stable-phase schizophrenia may comprise two distinct nosological entities namely Major Neuro-Cognitive Psychosis (MNP, largely overlapping with the deficit syndrome) and simple NP (SNP), which are defined by neuroimmune and neurocognitive abnormalities. Furthermore, cognitive impairments and PHEM (psychotic, hostility, excitation, mannerism) and negative symptoms load on the same dimension.

METHODS: The current study aimed to investigate associations of psychomotor retardation (PMR) and clinical as well as biomarker characteristics of schizophrenia. We recruited 40 healthy controls and 79 schizophrenia patients and measured IgA responses to tryptophan catabolites (TRYCATs), IgM to malondialdehyde and nitroso (NO)-cysteinyl, macrophage inflammatory protein-1 (MIP-1), soluble interleukin (IL)-1 receptor antagonist (sIL-1RA), IL-10, CCL-11 as well as PMR items of different rating scales and motor screening task (MOT).

RESULTS: PMR differentiated schizophrenia from controls and MNP from SNP. In addition, PMR was strongly associated with executive functions, deficits in episodic and semantic memory, PHEM and negative (PHEMN) symptoms. Around 50% of the variance in PMR was predicted by the cumulative effects of immune activation, CCL-11, TRYCATs and NO-Cysteinyl levels, and lowered natural IgM. PRM may be reliably combined with PHEMN symptoms and memory and executive impairments into one latent vector reflecting overall psychopathology.

CONCLUSIONS: Current findings indicate that PMR may be a key psychopathological feature of schizophrenia and mainly MNP. In addition, PMR and associated impairments in memory and executive functions, and PHEMN symptoms may be driven by deficits in the compensatory

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immune regulatory system (natural IgM) combined with increased production of neurotoxic immune products, namely TRYCATs and IgM to NO-cysteinyl, and an endogenous cognition deteriorating chemokine, namely CCL-11.

Key words: schizophrenia, inflammation, nitrosative stress, tryptophan catabolites, cytokines, oxidative stress

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Introduction

In 1995, the first author of this paper proposed that activated neuro-immune pathways contribute to the pathophysiology of schizophrenia through infection-associated neurodevelopmental trajectories and other immune hits culminating in neuroinflammation, damage by neuro-oxidative and neuro-nitrosative stress, and activation of indeoleamine 2,3-dioxygenase (IDO) leading to increased production of tryptophan catabolites (TRYCAT) (Smith and Maes 1995). Since then, accumulating evidence indicates that neuro-immune and neuro-oxidative pathways, including the TRYCAT pathway, affect brain neuronal circuits and neuroprogressive pathways, which ultimately lead to cognitive deficits and schizophrenia symptom dimensions (Anderson and Maes, 2013; Roomruangwong et al., 2018b; Kanchanatawan et al., 2018a; Davis et al., 2014; 2016).

Recently, based on new findings from our laboratory, we have proposed novel immune, phenomenological and cognitive models of schizophrenia (see **Figure 1**). The first model is described as the IRS (immune-inflammatory response system) - CIRS (compensatory immune regulatory system) model of schizophrenia (Roomruangwong et al., 2018b). The different subtypes of schizophrenia (including first-episode psychosis, treatment-resistant schizophrenia as well chronic schizophrenia) are accompanied by signs of IRS activation, as indicated by increased levels of acute phase proteins, complement factors and activated macrophagic M1, T helper (Th)-1 and Th-17 cells (Maes et al., 1994; 1997; Anderson and Maes, 2013; Roomruangwong et al., 2018; Noto et al., 2018). However, the same schizophrenia-related phenotypes are also characterized by an activation of immune-regulatory pathways, including activated Th-2 and Treg

immune subsets and an increased production of acute phase reactants (such as haptoglobin), TRYCATs, and cytokine receptors such as the soluble interleukin (IL)-2R, s-IL-1 receptor antagonist (sIL-1RA) and soluble tumor necrosis factor receptor (sTNF-R)1 and sTNF-R2, as well as lowered peripheral tryptophan levels (Roomruangwong et al., 2018b). These immune regulatory processes appear to be secondary to IRS activation and downregulate the primary IRS thereby attenuating overzealous inflammatory responses (Roomruangwong et al., 2018b). Furthermore, schizophrenia patients exhibit deficits in the CIRS as indicated by, for example, lowered plasma levels of Clara Cell secretory protein **(**CC16**)**, an endogenous disulfide**-**bridged protein with anti**-**inflammatory cytokine properties, and relative decrements in levels of sIL-2R, sIL-1RA, sTNF-R1 and sTNF-R2 (Maes et al**.**, 1996; 1997a; Noto et al**.**, 2018; Roomruangwong et al., 2018b). These findings indicate that different phenotypes of schizophrenia are accompanied by deficits in the CIRS and hence more prominent IRS responses following infections and other immune challenges **(**Noto et al**.**, 2018; Roomruangwong et al**.**, 2018b**).** Importantly, products derived from the concomitant activation of the IRS and CIRS, which are increased in schizophrenia, may exert cytotoxic, neurotoxic and excitotoxic effects on brain cells thereby promoting neuroprogression (Roomruangwong et al., 2018b). Not only M1 macrophagic products, including IL-1β, IL-6 and TNF-α, Th-1 products, including IL-2, interferon-γ and TRYCATs (such as picolinic acid (PA), xanthurenic acid (XA) and 3-OH-kynurenine (3-OHK), but also Th-2 related cytokines and chemokines, such as IL-4, IL-13 and CCL11 (eotaxin) may have detrimental effects and therefore play a role in the immunopathogenesis of schizophrenia (Roomruamgwong et al., 2018b; Kanchanatawan et al., 2018b; Sirivachyakul et al., 2019; Maes and Carvalho, 2018).

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The second phenomenological model developed by our laboratories shows that stable-phase schizophrenia may be conceptualized as two distinct nosological entities namely Major Neuro-Cognitive Psychosis (MNP) (largely overlapping with the deficit subtype) and Simple Neuro-Cognitive Psychosis (SNP) (Kanchanatawan et al., 2018c). Both MNP and SNP appear to be distinct phenotypes which are delineated by neuroimmune and neurocognitive impairments and symptom severity as well. Thus, MNP is discriminated from SNP by highly increased TRYCAT levels including PA, XA and quinolinic acid (QA), lowered levels of natural IgM to malondialdehyde (and other oxidative specific epitopes (OSEs) and increased IgM responses to nitroso (NO)-Cysteinyl (Kanchanatwan et al., 2018c; Maes et al., 2018). IgM antibodies to MDA and other oxidative self-antigens are produced by B1 cells and may be present even without previous antigenic contact. Most importantly, these natural IgM antibodies are part of the innate immune system, which has strong immune-regulatory, anti-inflammatory and anti-oxidant effects and therefore are an integral component of the CIRS (Maes et al., 2018). Increased levels of IgM directed to NO-Cysteinyl may reflect increased nitrosylation and IgM responses to NO-cysteinyl, which is neurotoxic and may cause demyelination (Maes et al., 2018).

Moreover, our findings indicate that psychotic, hostility, excitation, mannerism and negative (PHEMN) symptoms load to the same psychopathological dimension and that these symptoms are strongly associated with impairments in episodic and semantic memory (Kanchanatawan et al., 2018d). Biomarkers such as IgA responses to PA, XA and 3OHK, CCL-11, IgM to NO-Cysteinyl (all positively) and IgM to MDA (inversely) appear to have a particularly strong impact on negative symptoms and excitation (Kanchanatawan et al., 2018a; 2018d;

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Sirivichayakul et al., 2018; 2019; Maes et al., 2018). Therefore, we posited that lowered natural IgM along with increased neurotoxic products (PA, XA, QA, 3OHK, eotaxin, IgM to NO-Cysteinyl) could have neurotoxic effects, which may drive PHEMN symptoms and the MNP phenotype (Sirivichayakul et al., 2018; Maes et al., 2018).

Moreover, we also reformulated existing neurocognitive models of schizophrenia by incorporating executive functions and memory impairments in our IRS/CIRS models (Sirivichayakyl et al., 2018; 2019; Maes et al., 2018). The executive functioning network may control and mediate episodic and semantic memory performance thereby regulating learning processes and, hence, we have examined whether the effects of immune activation on memory and PHEMN symptoms could be mediated by executive functions. Our results showed that activated immune pathways with increased production of PA, XA, QA an 3-OHK (all putative neurotoxic mediators) and eotaxin which affects neurogenesis impact executive control, which, in turn, predicts the memory deficit syndrome characterized by dysfunctional learning processes and formal thought disorders (FTD), which subsequently may cause false memories and, therefore, PHEMN symptoms (Sirivichayakul et al., 2018; 2019).

Psychomotor retardation (PMR) with impairments in both fine and gross motor performance is another symptom cluster that characterizes schizophrenia (Morrens et al., 2007; Hamdioui and Lotfi, 2016; Walther and Strik, 2012). The presence of PMR in schizophrenia patients is indicated by slow motor responses, an extended response latency and slow movements and, in more extreme cases, catatonia (Morrens et al., 2007). This PMR symptom cluster is also referred to as bradykinesia, movement planning deficit or psychomotor poverty syndrome. Commonly used

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rating scales to assess severity of schizophrenia comprise PMR symptoms including motor retardation, decreased spontaneous movements, poverty of speech, blunting of affect and increased latency of response (Morrens et al. 2007). PM slowing in schizophrenia, as measured by psychomotor tasks, processing speed tests and drawing movements, is sometimes, but not always associated with negative symptoms, while correlations with positive symptoms are even more variable (Henkel et al., 2004; Morrens et al., 2007). There is also evidence that executive functions, particularly planning (but not set-shifting or spatial working memory), predict fine motor control (Riddle, 2013). However, there is no evidence to suggest that PMR in schizophrenia is modulated by executive functions, such as planning. In addition, associations of PMR and aforementioned immune biomarkers, PHEMN symptoms, memory deficits and the MNP (deficit) phenotype remain to be investigated.

Thus, the present study was conducted to examine whether PMR could be predicted by immune biomarkers and executive functions and whether PRM could be associated with memory, PHEMN symptoms and the MNP phenotype.

Methods

Participants

In this study we included 119 participants, namely 40 healthy controls and 79 patients with schizophrenia. The patients were recruited at the Department of Psychiatry, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. All patients complied with the diagnostic criteria of schizophrenia according to the DSM-IV-TR and all patients were in a stable phase of illness, i.e., they did not suffer from acute episodes the year prior to the study. In addition, we divided the

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schizophrenia patients into two groups, those with and without deficit schizophrenia (Kirkpatrick et al., 1989). We excluded schizophrenia patients who suffered from acute psychotic episodes the year prior to inclusion and those with axis-1 DSM-IV-TR disorders other than schizophrenia, including major depression, bipolar disorder, schizoaffective disorder, substance use disorders, and psycho-organic disorders.

Patients and controls were recruited from the same catchment area, namely province Bangkok, Thailand. Healthy volunteers were excluded when they showed a current or lifetime diagnosis of any axis I diagnosis according and when they had a positive family history of schizophrenia. Schizophrenia patients and controls were excluded when they presented with neuro-immune or neurodegenerative disorders including stroke, Parkinson′s disease, multiple sclerosis and Alzheimer′s disease, or medical illnesses such as psoriasis, rheumatoid arthritis, COPD, inflammatory bowel disease, and diabetes (type 1 and 2). We excluded patients and controls who had ever been using medications known to interfere with immune functions, such as glucocorticoids or immunosuppresiva, and those who took supplements with antioxidants or ω3-polyunsaturated fatty acids the months prior to the study. All controls and patients as well as the guardians (parents or close family members) of patients gave written informed consent prior to participation in our study. The study was conducted according to International and Thai ethics and privacy laws. Approval for the study (298/57) was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, which is in compliance with the International Guidelines for Human Research protection as required by the Declaration

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of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Measurements

Clinical assessments

The diagnosis of schizophrenia was made by a senior psychiatrist specialized in schizophrenia (BK) according to DSM-IV-TR diagnostic criteria using the Mini-International Neuropsychiatric Interview (M.I.N.I.), in a validated Thai translation (Kittirathanapaiboon and Khamwongpin, 2005). The same day as the M.I.N.I., the same senior psychiatrist used a semi-structured interview to assess clinical and socio-demographic data in patients and controls. We also made the diagnosis of a first episode versus multiple episodes (DSM-5). The same day BK also assessed the Schedule for the Deficit Syndrome (SDS) (Kirkpatrick et al., 1989), the Scale for the Assessments of Negative Symptoms (SANS) (Andreasen, 1989), the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) and the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). Schizophrenia patients were divided into two groups, namely MNP and SNP, using algorithms computed by unsupervised machine learning techniques as explained previously (Kanchanatawan et al. 2018d).

On the same day, different neuropsychological (NP) tests were assessed by a well-trained research assistant, who has a MSc degree in mental health. This research assistant was blinded to the clinical diagnosis and used the CANTAB (*Cambridge* Neuropsychological Test Automated

Battery) tests (CANTAB, 2018) and the CERAD-NP (Consortium to Establish a Registry for Alzheimer′s disease) (CERAD, 1986) batteries. In this study, we considered 4 CANTAB tests, namely a) Motor screening task (MOT) to screen gross motor function, namely motor speed and accuracy of movements. We analyzed 3 MOT tests, namely MOT mean latency (MOT-ML), which measures motor latency (time taken for the participant to touch a cross after it appears on a screen), MOT mean error (MOT_ME), which assesses accuracy of the participant′s pointing (expressed as the distance between where the participant touches the screen and the target, i.e. the centre of the cross), and MOT total correct (MOT_TC), which is the total number of correct responses during ten assessed trials); b) One touch stockings of Cambridge (OTS) to probe spatial planning. We tested the OTS probability solved on first choice (OTS_PSOFC); c) Rapid visual information process test (RVP), which probes visual sustained attention. In the current study, we used RVP A′ Prime (RVP_A) ″and RVP median latency (RVP_ML); and d) Spatial working memory (SWM), namely SWM between errors (SWM_BE) and SWM strategy (SWM_STR) to probe working memory and strategy use. As explained in detail elsewhere, we employed a latent vector (LV) extracted from three CANTAB tests reflecting executive functions (Sirivichayakul et al., 2019), namely OTS_PSOFC, SWM_BE and SWM_STR. As such this ″executive LV″ is a measure of executive functions, especially planning, working memory and strategy use combined. On the same day, the same research assistant also assessed different CERAD tests, namely a) the Mini-Mental State Examination (MMSE), which tests various functions including concentration, orientation, naming, memory and constructional praxis; b) Verbal Fluency Test (VFT) which

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assesses fluency and semantic memory; c) Word List Memory (WLM) to probe learning ability and verbal episodic memory; and d) Word List Recall, true recall (True Recall) to probe verbal episodic memory recall. We used DSM-IV-TR criteria to make the diagnosis of Tobacco Use Disorder (TUD). Body mass index (BMI) was assessed during the same day of the clinical interview and was scored as body weight (kg) / length (m^2).

Indices reflecting psychomotor retardation (PMR) were constructed using z-unit weighted composite scores based on items of the BPRS, HDRS, PANSS and SANS. A first PMR index1 (PMRI1) was computed as z-score of the HDRS item 8 (HDRS8: psychomotor retardation: slowness of thought and speech, decreased motor activity, impaired inability to concentrate) *plus* z-score of the general psychopathology scale of the PANSS, item G7 (zPANSSG7; reduction in motor activity as reflected in slowing or lessening of movements and speech, diminished responsivess to stimuli and reduced body tone) *plus* z score of item 13 of the BPRS (zBPRS13; reduction in energy level evidenced in slowed movements). The second PMR index2 (PMRI2) was computed as the sum of zHDRS8 + zG7 + zBPRS13 + zMOT_ML + z SANS1.2 (decreased spontaneous movements) + zSANS2.1 (poverty of speech) and zSANS2.4 (increased latency of response).

Based on PANSS and BPRS items we employed four z-unit weighted composite scores reflecting four different PHEM dimension scores (Kanchanatawan et al., 2018d). The severity of psychotic symptoms was computed as sum of zPANSS (positive subscale item 1) P1 (delusion) + zPANSSP3 (hallucinations) + zPANNSP6 (suspiciousness) + zBPRS11 (suspiciousness) +

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zBPRS12 (hallucinatory behavior) + zBPRS15 (unusual thought content). The severity of the hostility dimension was assessed as the sum of zPANSS7 (hostility) + zPANSSG14 (poor impulse control) + zBPRS10 (hostility) + zBPRS14 (uncooperativeness). The excitement dimension score was computed as zP14 (excitement) + zP5 (grandiosity) + zBPRS8 (grandiosity) + zBPRS17 (excitement). Mannerism was computed as zG5 + zBPRS7 (both mannerism and posturing). FTD and abstract thinking (FTD) was computed as z value of PANNS P2 (item P2 of the PANNS scale or conceptual disorganization, zP2) + zN5 (item N5 of the PANNS or difficulty in abstract thinking) + zBPRS4 (item 4 of the BPRS or conceptual disorganization) (Sirivichayakul et al. 2019).

Assays

In patients and controls, fasting blood was sampled at 8.00 a.m. for the assay of IgM-mediated autoimmune responses directed against MDA and NO-cysteinyl, IgA response to TRYCATs and 4 cytokines/chemokines, namely CCL-11, IL-10, sIL-1RA and MIP. An enzyme-linked immunosorbent assay (ELISA) was used to measure IgM levels directed against conjugated MDA (Daverat et al., 1989; Boullerne et al., 1996; Amara et al., 1995; Faiderbe et al., 1992). MDA was linked to fatty acid free-bovine serum albumin (BSA), according to previously described methods. Synthesis of the conjugates to delipidated BSA was performed as described before (Amara et al., 1995). In order to mimic nitrosylation processes, NO-cysteinyl was synthesized by linking haptens to BSA (Sigma-Aldrich) using glutaraldehyde (Boullerne et al., 1996; Geffard et al., 2003; Boullerne et al., 1995). The synthesis of these conjugates has been described previously

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(Boullerne et al., 2002). The hapten conjugate was nitrosylated using sodium nitrite ($NaNO_2$) dissolved in 2 ml of each conjugate, in 0.5 M HCl at 37°C for 2 h, while shaking in the dark. The conjugate was then dialyzed at 4°C for 24 h against a Phosphate Buffered Saline (PBS: 10^{-2} M NaH_2PO_4, $12H_2O$; 0.15M NaCl; pH 7.4) solution. S-nitrosothiol bond formation was determined by spectrophotometry. The S-nitrosothiol compound possesses two absorbance maxima, at 336 and 550 nm, respectively: e_{336} nm= 900 $M^{-1}cm^{-1}$ for the conjugates, e_{550}nm = 4000 $M^{-1}cm^{-1}$ for BSA. Absorbance was evaluated in order to determine NO concentrations linked to the compound. The detection of IgM autoantibodies to the conjugates was performed by indirect ELISA tests (Faiderbe et al., 1992; Boullerne et al., 2002). Briefly, polystyrene 96-well plates (NUNC) were coated with 200 µl solution containing the conjugates or BSA in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4°C for 16 h under agitation. Then, a 200 µl of blocking solution (PBS, 2.5 g/l BSA) was added for 1 h and placed at 37°C. Following three washes with PBS, plates were filled up with 100 µl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 10% Glycerol, 2.5 g/l BSA, 1 g/l BSA-G) and incubated at 37°C for 2 h. After three washes with PBS-0.05% Tween 20, plates were incubated at 37°C for 1 h with peroxidase-labeled anti-human IgM secondary antibodies diluted respectively at 1: 15,000, in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). They were then washed three times with PBS-0.05% Tween 20, and incubated with the detection solution for 10 min in the dark. Chromogen detection solution was used for the peroxidase assay at 8% in 0.1 M acetate and 0.01 M phosphate buffer (pH 5.0) containing 0.01% H_2O_2. The reaction was stopped with 25 µl 2-N HCl. ODs were measured at 492

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nm using a multiscan spectrophotometer. All assays were carried out in duplicate. The intra-assay coefficients of variation (CV) were < 6%.

The TRYCATs were assayed as described previously (Roomruangwong et al., 2017; 2018a; Duleu et al., 2010). The 6 TRYCATs were dissolved in 200 μL dimethylsulfoxide (DMSO) (Acros). Bovine serum albumin (BSA) (ID Bio) was dissolved in 3mL 2-morpholino-ethanesulfonic acid monohydrate (MES) buffer 10^{-1} M at pH = 6.3 (Acros). The TRYCATs were then mixed with the BSA solution and supplemented with 15mg N-hydroxysuccinimide (Sigma) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (Acros) as coupling agents. The conjugates were synthesized by linking 3-OH-kynurenine (3HK) (Sigma), kynurenic acid (KA) (Acros), quinolinic acid (QA) (Acros), anthranilic acid (AA) (Acros), xanthurenic acid (XA) (Acros) and picolinic acid (PA) (Acros) to 20 mg BSA. The coupling reaction proceeded at 37°C for 1 hour in the dark. The coupling was stopped by adding 100 mg hydroxylamine (Sigma-Aldrich) per conjugate. Protein conjugates were dialyzed with 10^{-1} M NaCl solution for 72 hours and the bath solution was changed at least four times per day. The conjugated TRYCATs and BSA concentrations were evaluated by spectrophotometry. The coupling ratio of each conjugate was determined by measuring the concentration of TRYCATs and BSA at 310–330nm and 280 nm, respectively. ELISA tests were used to determine plasma titers of immunoglobulins (Ig) A (IgA). Towards this end, polystyrene 96-well plates (NUNC) were coated with 200 μL solution containing 10–50 μg/mL TRYCAT conjugates in 0.05Mcarbonate buffer (pH = 9.6). Well plates were incubated under agitation at 4°C for 16 hours. Then, 200 μL blocking buffer A (Phosphate

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Buffered Saline, PBS, 2.5 g/L BSA) was applied and all samples were incubated at 37°C for 1 hour. Well plates were washed with PBS solution and filled up with 100 μL serum diluted 1:130 in blocking buffer and incubated at 37°C for 1 hour and 45 minutes. Well plates were washed 3 times with PBS, 0.05% Tween 20, incubated with peroxidase-labeled goat anti-human IgA (SouthernBiotech) antibodies at 37°C for 1 hour. The goat anti-human IgA antibody was diluted at 1:10.000 in blocking buffer (PBS, 2.5 g/L BSA). Plates were then washed three times with PBS, 0.05% Tween 20. Fifty microlitre of TMB substrate (3,3',5,5'-Tetramethylbenzidine, SouthernBiotech) was added and incubated for 10 minutes in the dark. The reaction was stopped using 50 µL of TMB stop solution (SouthernBiotech). Optical densities (ODs) were measured at 450 nm using Varioskan Flash (Thermo Scientific). All assays were carried out in one and the same run by the same operator (SS) who was blind to all clinical results. All assays were carried out in duplicate. The analytical intra-assays CV values were < 7%. We computed a noxious / protective TRYCAT ratio as z score of PA (zPA) + zXA + z3OHK − zAA − zKA (NOX/PRO ratio).

For cytokines/chemokines, 50 µl of serum (1:2 dilution in calibrator diluent) was mixed with 50 µl of microparticle cocktail containing CCL-11, sIL-1RA, IL-10 and MIP-1α (R&D Systems, Inc, Minneapolis, MN, USA) per well of a 96-well plate provided by manufacturer and incubated for 2 hours at room temperature on a shaker at 800 rpm. The mixture was then washed 3 times with wash buffer and 50 µl diluted Biotin Antibody cocktail was added and then incubated for 1 hour. Wells were washed 3 times before another 50 µl of diluted Streptavidin-PE was added

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and further incubated for 30 minutes. Finally, wells were washed 3 times and 100 µl of wash buffer was added and left at room temperature for 2 minutes before being read with Bio-Plex® 200 System (Bio-Rad Laboratories, Inc.). The intra-assay CV values were <7.0%. The least detectable dose was 1.82 pg/mL for eotaxin, 1.58 pg/mL for MIP-1, 5.98 pg/mL for IL-1RA and 0.4 pg/mL for IL-10. We computed an immune activation index as z score of interleukin-10 (zIL-10) + zMIP + zsIL-1RA.

Statstical analysis

We used analysis of variance (ANOVA) to check differences in continuous variables between categories and analysis of contingency tables (X^2-test) to assess associations between categorical variables. Correlation matrices were computed to check correlations between MOT_ML, both PMR indices and clinical and biomarker data using Pearson′s product moment, point-biserial and Spearman′s rank order correlation coefficients. We used multiple regression analysis to assess the most significant biomarkers and CANTAB variables that predict PMRI1, PMRI2 and MOT_ML. Receiver Operating Characteristics (ROC) analyses were employed to compute the area under the ROC curve. Results of multiple regression analyses were checked for multicollinearity. All analyses were bootstrapped (n=1000) and we report differences (if any) between results with and without bootstrapping. IgM to MDA, eotaxin, IL-10, sIL-1RA, MIP, MOT_ML and MOT_ME values were processed in Ln transformations in order to normalize the data distribution of these variables. Tests were 2-tailed and a p-value of 0.05 was used for statistical significance.

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Multilayer perceptron (MLP) Neural Network (NN) models were employed to assess the complex associations between diagnosis of MNP and schizophrenia (output variables) with as input variables schizophrenia symptoms including the PMR indices. We used automated feedforward architecture models to train the network and employed two hidden layers with up to 6 nodes and used minibatch training with gradient descent and 30 epochs. 46.67% of all participants were randomly allocated to a training set (in order to estimate the network parameters), 20.0% of the cases to a testing set (to prevent overtraining) and 33.33% to a holdout sample (to evaluate the final network). One consecutive step with no further decrease in the error term was used as stopping rule. Finally, we computed error, relative error and importance for all input variables. All abovementioned statistical analyses were performed using IBM SPSS windows version 25.

Partial Least Squares path modeling (SmartPLS) (Ringle et al., 2014) was used to decipher possible causal links between biomarkers (the immune activation index, eotaxin, NOX/PRO ratio, IgM to MDA and NO-Cysteinyl), and symptom dimensions, including the PMR indices and PHEMN symptoms, whereby it is considered that memory and executive deficits may mediate these associations. The variables examined in the path models were entered as indicator variables (e.g. the biomarkers) or as latent vectors (LV) extracted from a set of indicator variables (e.g. the PMR LV and PHEMN LV). PLS path modeling was only performed when the latent constructs and overall model complied with specific quality criteria, namely a) all indicators of the LVs have factor loadings > 0.400 with $p<0.001$; b) the overall quality of the model as assessed with SRMR should be < 0.08; c) latent constructs should have a good reliability as indicated by Cronbach′s

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alpha > 0.7, composite reliability > 0.7, rho_A > 0.80 and average variance extracted (AVE) > 0.500; and d) construct crossvalidated redundancies and communalities should be adequate (Ringle et al., 2014). Subsequently we used complete, consistent bootstrapping (2000 bootstraps) to compute path coefficients with p-values, and total, total indirect and specific indirect effects.

Results.

1. Construction of two psychomotor retardation indices.

Figure 2 shows how the two PMR indices were constructed, namely PMRI1 and PMTI2. The first PMRI1 was constructed by entering HDRS8, PANSSG7 and BPRS13 as three indicators of a PMRI LV in the PLS analysis. The second was constructed using these three indicators coupled with MOT_PL, SANS1.2, SANS2.1 and zSANS2.4. **Table 1** shows the reliability characteristics of these two LVs. The "PMRI1 LV" performed well as a composite score showing adequate AVE, composite reliability, Cronbach's alpha and rho_A values. All three indicators loaded highly (>0.567) and significantly (<0.0001) on this LV. Table 1 also shows that the PMRI2 LV showed an adequate reliability with a AVE, composite reliability and rho_A. In both indices BPRS13 showed the lowest loadings on the LVs. There was a highly significant correlation between both PMRI1 and PRMI2 scores ($r=0.932$, $p<0.001$, $n=119$).

2. Associations between PMR indices and neuropsychological functioning.

In order to validate PMRI1 as a motor concept and to assess the associations between both indices and executive functions and sustained attention we have examined the associations

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between PRMI1 and MOT_ML and executive CANTAB tests including SWM_BE, SWM_STR and OTS_PSOFC, and between PMRI2 and the same CANTAB variables. Table 2 shows the results of multiple regression analyses with the PMR indices as dependent variables and the CANTAB tests as explanatory variables. We found that 24.0% of the variance in PMRI1 was explained by the regression on MOT_ML, RVP_ML and OTS_PSOFC, while 27.4% of the variance in PMRI2 was explained by RVP_ML and SWM_BE.

PMRI1 was significantly correlated with MOT_ML ($r=0.432$, $p<0.001$, all $n=119$), RVP_A ($r=-0.371$, $p<0.001$), RVP_BE ($r=0.272$, $p=0.003$), SWM_BE ($r=0.357$, $p<0.001$), SWM_STR ($r=181$, $p=0.049$), OTS_PSOFC ($r=-0.390$, $p<0.001$), MMSE ($r=-0.478$, $p<0.001$), VFT ($r=-0.514$, $p<0.001$), WLM ($r=-0.616$, $p<0.001$), and True Recall ($r=-0.610$, $p<0.001$). There was no significant correlation between PMRI1 and MOT_ME ($r=-0.073$, $p=0.433$). PMRI2 was significantly correlated with RVP_A ($r=-0.478$, $p<0.001$), RVP_BE ($r=0.304$, $p=0.001$), SWM_BE ($r=0.414$, $p<0.001$), SWM_STR ($r=197$, $p=0.032$), OTS_PSOFC ($r=-0.423$, $p<0.001$), MMSE ($r=-0.537$, $p<0.001$), VFT ($r=-0.560$, $p<0.001$), WLM ($r=-0.651$, $p<0.001$) and True Recall ($r=-0.659$, $p<0.001$).

3. PMR indices and schizophrenia symptoms and classification

We found significant correlations between PMRI1 and SDS ($r=0.870$, $p<0.001$), SANS ($r=0.833$, $p<0.001$), FTD ($r=0.536$, $p<0.001$), psychosis ($r=0.700$, $p<0.001$), hostility ($r=0.413$, $p<0.001$), excitation ($r=0.782$, $p<0.001$) and mannerism ($r=0.520$, $p<0.001$). PMRI2 was significantly associated with the total SDS score ($r=0.931$, $p<0.001$), FTD ($r=0.531$, $p<0.001$),

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psychosis (r=0.682, p<0.001), hostility (r=0.450, p<0.001), excitation (r=0.785, p<0.001) and mannerism (r=0.552, p<0.001). The MOT_ML scores were significantly associated with SDS total score (r=0.485, p<0.001), SANS (r=0.510, p<0.001), FTD (r=0.298, p=0.001), psychosis (r=0.364, p<0.001), hostility (r=0.262, p=0.004), excitation (r=0.395, p<0.001) and mannerism (r=0.368, p<0.001).

In order to check whether the PMR indices are key characteristics of MNP and schizophrenia we have performed neural network analyses with diagnosis (either MNP versus SNP and controls or schizophrenia versus controls) as output variables and the PMR indices as explanatory variables. We also entered other symptoms as input variables, namely psychosis and excitement (Kanchanatawan et al., 2018d), FTD (Sirivachyakul et al., 2019) and two SANS subscores, i.e. attention and anhedonia, which do not reflect psychomotor slowing (flattening alogia and apathy are related to PMR). Table 3 shows the network information of model 1 examining the separation of schizophrenia versus controls. The first network was trained using 2 hidden layers with 4 and 3 units in layer 1 and 2, respectively, and with hyperbolic tangent as activation function in hidden layer 1 and identity in the output layer. The differences in sum of squares error between the training and testing sets indicate that the model has learnt to generalize from the trend. The rate of (in)correct predictions was fairly constant in the training, testing and holdout sample showing that the model is not overfitted. Table 3 displays the partitioned confusion matrices showing an accuracy of 96.3% in the holdout sample with a sensitivity of 94.7% and a specificity 100.0%. **Figure 2** shows the relative importance of all input variables. Anhedonia,

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PMRI2, FTD, psychosis and excitement are the most important determinants of the predictive power of the model, while attention follows at a distance.

Table 3 shows the results of the second NN model with MNP versus no-MNP as output variables and the same input variables as model 1. The second network was trained as the first one with 2 hidden layers with 4 and 3 units in layer 1 and 2, respectively, and with hyperbolic tangent as activation function in hidden layer 1 and identity in the output layer. The differences in sum of squares error between the training and testing sets and the rate of (in)correct predictions in the training, testing and holdout samples shows that the model learnt to generalize from the trend and that the model is not overtrained. The results of the partitioned confusion matrix shows an 100% accuracy rate in the holdout sample. **Figure 3** shows that the importance chart is dominated by anhedonia and PMRI2, while attention and FTD are relevant determinants which follow at a distance.

ANOVAs showed significantly higher PMRI1 ($F=36.08$, $df=1/76$, $p<0.001$), PMRI2 ($F=68.46$, $df=1/76$, $p<0.001$) and MOT_ML ($F=8.86$, $df=1/76$, $p=0.004$) scores in MNP versus SNP, but no significant differences in MOT_ME ($F=0.22$, $df=1/76$, $p=0.639$) and MOT_TC ($F=3.03$, $df=1/76$, $p=0.086$) scores. ANOVAs showed that there were no significant differences in PMRI1 ($p=0.435$), PMRI2 ($p=0.355$), MOT_ML ($p=0.245$) and MOT_TC ($p=0.526$) between subjects with a first versus patients with multiple episodes.

We have also examined possible effects of drug state on both PMR indices and the MOT test results as well. We could not find any significant effect of use of risperidone (n=34), clozapine (n=10), haloperidol (n=11), perphenazine (n=21), antidepressants (n=26), mood stabilizers

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(n=13) and anxiolytics / hyponotics (n=29) on the PMR indices or MOT measurements, not even at the p=0.05 level (without p-correction for multiple testing). Previously, we have shown that the same drugs gave no effect on CCL-13, IgM to MDA and NO-cysteinyl and TRYCAT levels (Maes et al., 2018; Sirivichayakul et al., 2018).

4. Association between PMRI-groups and socio-demographic and clinical data

Using the median-split method applied on PMRI1 scores we divided the schizophrenia sample into two groups, namely patients with and without increased PMRI1 scores. **Table 4** shows the demographic and clinical differences between controls and patients with lower and higher PMRI1 scores. Patients with increased PMRI1 scores showed higher MOT_ML, SDS, SANS, psychosis, hostility, excitation, mannerism and HDRS scores as compared with those with lower PMRI1 scores and controls. The MOT_ME and MOT_TC scores were not significantly different among the study groups. Years of education was somewhat lower in the high-PMRI1 group as compared with controls. There were no significant differences in socio-demographic data, duration of illness and first versus multiple episodes between both patient groups. There was a significant association between the PMRI1-groups and the diagnosis of deficit schizophrenia and MNP. **Table 5** shows the cognitive test results in the three study samples. Patients with PMRI1 had significantly worse test results on the MMSE, VFT, WLM, WL Recall, RVP_A, than patients with lower PMRI1 scores and controls.

5. Associations among PMR indices and biomarkers

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Table 5 shows that IgM to MDA was significantly lower in patients with high PMRI1 scores than in the other groups. We found significant correlations between the PMRI1 and IgM to MDA (r=-0.389, p<0.001), immune activation index (r=0.327, p<0.001), CCL-11 (r=0.391, p<0.001) and IgA NOX/PRO (r=0.332, p<0.001). There were also significant correlations between PMRI2 and IgM to MDA (r=-0.458, p<0.001), immune activation index (r=0.336, p<0.001), CCL-11 (r=0.378, p<0.001) and IgA NOX/PRO (r=0.358, p<0.001). The MOT_ML scores were significantly correlated with IgM to MDA (r=-0.286, p=0.002), immune activation index (r=0.244, p=0.007), CCL-11 (r=0.290, p=0.001) and IgA NOX/PRO (r=0.248, p=0.006).

Table 6 shows that 39.7% of the variance in PMRI1 was explained by the regression on IgM MDA (inversely), IgM to NO-Cysteinyl, CCL-11 and IgA NOX/PRO (all positively). Up to 43.4% of the variance in PMRI2 was explained by the regression on IgM MDA (inversely), IgM NO-Cysteinyl, CCL-11 and IgA NOX/PRO (all positively). A large part of the variance (17.0%) in MOT_ML was explained by the cumulative effects of IgM to MDA, immune activation and CCL-11. Also, in the restricted sample of schizophrenia patients, a large part of the variance (28.4%) in PMRI1 was explained by IgM to MDA and IgM to NO-Cysteinyl (F=11.55, df=2/76, p<0.001), while the same variables explained 36.6% of the variance in PMRI2 (F=21.94, df=2/76, p<0.001). In schizophrenia, the same variables explained 12.8% of the variance in MOT_ML (F-5.68, df=2/77, p=0.005).

Results of PLS analyses

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In **Figure 5** we examine the causal links between the biomarkers (explanatory variables) and executive LV (entered as an indicator variable), memory (a LV extracted from FTD, True Recall and WLM), PHEMN symptoms (LV extracted from SDS total score and the 4 PHEM scores), and PMRI2 (LV extracted from the 7 indicators as described in Table 1). Moreover, in accordance with the schizophrenia model described in the Introduction, immune activation predicted eotaxin and TRYCAT levels and the executive LV which predicted the memory LV, PMRI2 and the PHEMN LV. Doing so, we used a multistep path model (namely from immune activation to CCL-11 and TRYCATs → executive functions → PMRI2, memory and PHEMN symptoms) with multiple mediators (including executive functions and memory) (Cepeda-Carrion et al., 2018). The overall model fit was good (SRMR=0.046), and the construct reliability and discriminant validity of the 4 latent constructs were good to excellent, namely all Cronbach′s alpha > 0.783, composite reliability > 0.792, rho_A > 0.790 and average variance extracted > 0.591, while all outer loadings of the 3 LVs showed loadings > 0.482 (all $p<0.001$). We found that 50.0% of the variance in PRMI2 was explained by executive functions, natural IgM, IgM NO-cysteinyl and CCL-11, and that 84.4% of the variance in PHEMN LV was explained by executive control. 45.7% of the variance in the executive LV was explained by CCL-11 and TRYCATs. Immune activation predicted both CCL-11 and TRYCAT levels, while immune activation was predicted by natural IgM. There were significant direct effects of immune activation on PMRI LV ($t=2.24$, $p=0.026$), memory LV ($t=3.60$, $p<0.001$) and symptom LV ($t=3.60$, $p<0.001$). There were also total direct effects of CCL-11 on PRM LV (3.26, $p=0.001$), memory LV ($t=3.62$, $p<0.001$) and PHEMN LV ($t=3.61$, $p<0.001$). There were also total direct effects of IgM NO-cysteinyl on PRM

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LV (2.98, p=0.003), memory LV (t=2.31, p=0.022) and PHEMN LV (t=2.27, p=0.024). Natural IgM showed total direct effects on PRM LV (t=6.55), memory LV (t=4.09) and PHEMN LV (t=3.97, all p<0.001). Also the TRYCATs significantly predicted PRM LV (t=2.99, p=0.003), memory LV (t=3.11, p=0.002) and PHEMN LV (t=3.20, p=0.001). The effects of immune activation on PMRI LV are mediated by the path from TRYCATs to executive functions (t=2.07, p=0.038) and specific indirect effects of TRYCATs on PMR LV mediated by executive functions (t=2.72, p=0.007).

Figure 6 shows a second PLS analysis with all indicators of PHEMN LV, executive LV and memory LV and PMRI2 combined in one LV. The model quality data were adequate with an SRMR = 0.054, while all loadings on this LV were significant (p<0.0001), namely PMRI2: 0.864, psychosis: 0.860, hostility: 0.689, excitement: 0.887, mannerism: 0.764, SDS: 0.865, FTD: 0.726, VFT: 0.636, WLM: 0.806, True Recall: 0.769 and executive control: 0.694. Moreover, this LV performed well as a composite score showing adequate AVE (0.562), composite reliability (0.932), Cronbach′s alpha (0.936) and rho_A values (0.941). We found that 52.7% of the variance in the LV extracted from these 11 indicators was explained by the combined effects of natural IgM to MDA, CCL-11, IgM NO-Cysteinyl, TRYCATs and education. There was a significant total effect of immune activation on this overall LV (t=4.00, p<0.001) and a specific indirect effect of immune activation, which was mediated by TRYCATs (t=2.63, p=0.009).

Discussion

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The first major finding of this study is that motor speed as measured with MOT_ML and PMR indices (but not motor accuracy) were significantly more impaired in patients with schizophrenia than in controls and more in patients with MNP (largely overlapping with deficit schizophrenia) than in those with SNP. Moreover, MOT_ML scores could reliably be combined with clinical ratings of slowed movements into valid composite scores, namely the PMR indices constructed here. MOT_ML probes speed and response latency, while the MOT_ME and MOT_TC assesses accuracy and the amount of errors. As such, slowed motor speed or increased response latency (as measured with the MOT_ML) is strongly associated with clinical ratings of slowing or lessening of spontaneous movements and speech, increased latency or response, poverty of speech, reduction in energy level, diminished responsivess to stimuli and reduced body tone. These findings indicate that the clinical ratings of PMR are validated and reflect sensorimotor impairments and, thus, that schizophrenia and especially MNP is accompanied by lowered PM speed and increased response latency rather that impairments in movement accuracy.

These findings extent those of previous studies showing that schizophrenia patients often (but not always) show objective signs of PMR (Morrens et al., 2007; Grootens et al., 2009). For example, Fuller and Jahanshahi (1999) reported that, using the plegboard, patients are slower than controls. Carnahan et al. (1997) observed a movement-planning deficit in schizophrenia patients, whilst Jogems-Kosterman et al. (2001) found that movement initiation and execution were slowed in schizophrenia patients. Decreased gait velocity and stride length were reported by Putzhammer et al. (2004). Nevertheless, some other authors could not find any differences in PM tests between schizophrenia patients and controls (Schroder et al., 1999). Braw et al. (2008) found greater deficits

in psychomotor speed in multi-episode schizophrenia patients than in first episode patients. In the current study, however, we could not find any differences in MOT and PMR indices between patients who suffered from one psychotic episode versus those with multi-episodes.

Some of these controversial results may be explained by the failure of previous studies to examine MNP or the deficit syndrome in PMR studies. Indeed, here we detected highly increased PMR ratings as well as MOT_ML scores in MNP as compared with SNP patients (and controls). For example, the difference in mean MOT_ML scores between MNP and SNP is 0.64 standard deviations (SDs), while that in PMRI1 and PMRI2 is 1.14 and 1.42 SDs, respectively. The differences between MNP and controls are even more pronounced, namely 1.1 SDs for MOT_ML, 1.88 for PMRI1 and 1.87 SDs for PMRI2. Also, our neural network analyses showed that PMR is a key component of schizophrenia and especially MNP. In this respect, it is interesting to note that Malla et al. (1995) found that (using Fitt′s task with a graphic tablet) the disorganization syndrome was associated with increased reaction times. Importantly, we found highly significant associations between severity of negative symptoms (the hallmark of MNP) and MOT_ML or PMR indices. Previously, Henkel et al. (2004) and Holthousen et al. (1999) reported a significant correlation between PM slowing and negative symptoms, although there are also controversial reports (Morrens et al., 2007). The highly significant associations between PMR indices and negative symptoms in our study could in part be explained by the inclusion of patients with MNP. In addition, we used items of the SANS to compute PMRI2, including decreased spontaneous movements, poverty of speech and increased latency of response (Morrens et al., 2007). Furthermore, many more SANS items are reminiscent of PMR, including (and not limited to)

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unchanged facial expression, paucity of expressive gestures, lack of vocal inflections, physical anergia and apathy. In addition, PMR not only causes slowing of physical responses, but also emotional responses including affect (Frith, 1995). Nevertheless, our PMRI1 score did not include any negative symptoms, but was based on psychomotor retardation (HDRS), reduction in motor activity (general subscale of the PANSS) and reduction in energy level as evidenced in slowed movements (BPRS). In our study, there were significant associations between PMR scores or MOT_ML and psychosis, hostility, excitation and mannerism, although the shared variance was lower than that between PMR and negative symptoms. A review of the literature shows that there may be a weak association between PM slowing and positive symptoms (Morrens et al., 2007; Fuller and Jahanshahi, 1999). Kontaxaki et al. (2014) reported that psychomotor ability is significantly associated with delusions and with restricted affect.

The second major finding of this study is that MOT_ML and PMR indices were significantly associated with MMSE, semantic and episodic memory and sustained attention scores. Our results extent those of Brebion et al. (2000) who reported that avolition and slowing of processing speed are associated with memory performance and these authors suggested that lowered memory performance in schizophrenia may be mediated by PMR. In contrast, other research found that factors based on processing speed and attention could be separated from motor (skill) factors (Woodward et al. 2005; Hobart et al., 1999). Another factor-analysis study showed that neurocognitive functions in schizophrenia consisted of different factors, including one representing motor functioning, a second processing speed, a third executive performance and a fourth verbal learning and memory (Bilder et al., 2002). Another factor analysis study showed the

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existence of three factors, namely psychomotor speed, cognition and attention, and verbal processing and memory (Mahurin et al., 1998).

In the current study, we found significant associations between MOT_ML and PMR indices and executive functions, especially planning and spatial working memory. Previously, it was shown that Trail Making Test (TMT) scores were significantly associated with the withdrawal and retardation factor of the BPRS and with psychomotor speed (Mahurin et al., 2006). Importantly, Riddle (2013) reported that executive functions, especially planning may predict fine motor control, suggesting that when planning abilities decline also motor control declines. Corti et al. (2017) reviewed that planning may be a compensatory resource for fine motor control in adults. It is known that executive engagement improves motor performance in older adults (Heuninckx et al., 2008; Seidler et al., 2010). The effects of planning on motor functions are supported by findings in schizophrenia that planning dysfunctions contribute to psychomotor slowing (Jogems-Kosterman et al., 2001). Leisman et al. (2016) suggest that cognitive processes (e.g. planning) underpin motor output, including intended and actual movement and named this effect "Motor-Cognition". The latter processes are localized in the M1 area, the premotor area, the presupplementary motor area (preSMA) and supplemetary motor area (SMA), which allow for motor planning, while the prefrontal cortex and basal ganglia initiate and organize the actions (Leisman et al., 2016). This explains that disorders in the premotor cortices are associated with aberrations in initiation of movements (Walther and Strik, 2012). The main function of the prefrontal cortex is the temporal organization of speech, behavior and reasoning with participation of working memory, orientation for action and control of interference (Fuster, 2009; Barbas, 2009).

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It should be underscored that executive functions also control semantic and episodic memory (Sirivichayakul et al., 2018), explaining in part the strong intercorrelations between executive functions, PMR and memory deficits reported here.

The third major finding of this study is that MOT-ML scores and PMR indices are highly significantly associated with neuro-immune biomarkers, i.e. 50% of the variance in PMR is predicted by the cumulative effects of increased immune activation, and higher CCL-11, TRYCATs and NO-Cysteinyl levels, coupled with lowered natural IgM directed to MDA and that these neuroimmune effects were in part mediated via executive functions. Moreover, we found that these biomarkers explained a large part of the variance in memory impairments and FTD, which in part was mediated by executive functions, and that the same biomarkers affected PHEMN symptoms and that the latter effects were completely mediated by memory functions. Previously, it was shown that psychomotor retardation in major depression is significantly associated with increased levels of haptoglobin, an acute phase reactant, indicating IRS activation (Maes et al., 1993). Recently, it was reported that in depression increased IL-6 levels are associated with psychomotor slowing (Goldsmith et al., 2016). Nevertheless, our findings show that in schizophrenia, deficits in the CIRS (lower natural IgM), and thus lowered immune regulation and anti-inflammatory effects (Maes et al., 2018), combine with the effects of increased CCL-11 (lowered neurogenesis), PA, XA, QA, 3OHK and IgM to NO-Cysteinyl (all neurotoxic) to bring about executive and memory dysfunctions, PMR and PHEMN symptoms (Kanchanatawan et al., 2018a; 2018d; Maes et al, 2018; Sirivichayakul et al., 2018; 2019). As such, PMR, slow motor speed, impairments in executive and memory dysfunctions and PHEMN symptoms have a

common pathophysiology, explaining that those factors could reliably be combined into one latent structure with excellent composite reliability (e.g. Cronbach α=0.936) and that a combination of our biomarkers had a strong impact on this latent vector. This indicates that the distinction between PMR, cognitive functions and psychopathology is rather artificial because these components belong to the same phenomenological dimension.

Our results suggest that lowered CIRS functions are accompanied by exaggerated responses to immune challenges leading to increased neurotoxicity and attenuated neurogenesis and that these pathways may explain the more widespread impairments in brain neurocircuitry underpinning executive functions, memory and psychomotor slowing. Structural or functional aberrations in the brain areas underpinning these cognitive and PM impairments also characterize schizophrenia, namely impairments in prefrontal system and frontal lobes, primary and SMA and preSMA cortices, and prefrontal-limbic and cortico-striatal loops that connect the prefrontal cortex with the basal ganglia, thalamus, hypothalamus, hippocampus and amygdala (Walther and Strik, 2012; Rabitt and Lowe, 2000; Liu et al., 2003; Fuster, 2009).

The results of our study should be interpreted considering its limitations. First, the cross-sectional design of the current study precludes the establishment of firm causal inferences. In addition, possible confounding variables such as metabolic comorbidities could have influenced some of the findings herein reported.

In conclusion, our findings indicate that PMR is a core manifestation of schizophrenia and mainly of the MNP or the deficit syndrome, which was found to be associated with PMR along with impairments in memory and executive functions and PHEMN symptoms. These different components of schizophrenia phenomenology may be driven by deficits in the CIRS (lowered

natural IgM), which combined with an increased production of neurotoxic compounds (including TRYCATs and IgM to NO-Cysteinyl) and an endogenous cognition deteriorating chemokine (CCL-11), may deleteriously impact neuroplastic mechanisms.

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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 manuscript. MM and BK designed the study. BK recruited patients and completed diagnostic interviews and rating scale measurements. MM carried out the statistical analyses. All authors (BK, MM, SS and AC) contributed to interpretation of the data and writing of the manuscript. All authors approved the final version of the manuscript.

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Table 1. Construct reliability and validity of the two psychomotor retardation indices (PMRI) constructed in the current study.

Features of the latent constructs		**PMRI1 items**	**PMRI2 items**
Variables	BDRS13	0.567	0.433
	G7	0.935	0.926
	HDRS8	0.953	0.913
	SANS1.2	-	0.851
	SANS2.1	-	0.893
	SANS2.4	-	0.883
	MOT_ML	-	0.624
Composite reliability		0.870	0.925
Cronbach α		0.775	0.899
Rho_A		0.894	0.929
Average variance extracted		0.704	0.653

BDRS13: Brief Psychiatric Rating Scale, item 13; reduction in energy level evidenced in slowed movements

G7: PANSS (Positive and Negative Syndrome Scale, General subscale), item 7: reduction in motor activity as reflected in slowing or lessening of movements and speech, dominished responsivess to stimuli and reduced body tone)

HDRS8: Hamilton Depression Rating Scale, item 8: psychomotor retardation: slowness of thought and speech, decreased motor activity, impaired inability to concentrate

SANS1.2: Scale for the Assessments of Negative Symptoms, item 1.2: poverty of speech

SANS2.1: SANS, item 2.1: decreased spontaneous movements

SANS2.4: SANS, item 2.4: increased latency of response

MOT_ML: motor screening task, mean latency

Table 2. Results of multiple regression analysis with the psychomotor retardation (PMR) scores as dependent variables and executive and attention neurocognitive scores as explanatory variables.

Dependent Variable	Explanatory Variables	B	SE	t	p	F model	df	p	Partial Eta squared
PMRI1	MOT_ML	0.288	0.112	2.57	0.012	11.71	3/111	<0.001	0.240
	RVP_ML	0.234	0.106	2.22	0.028				
	OTS	-0.187	0.090	-2.08	0.040				
PMRI2	RVP_ML	1.95	0.517	3.78	<0.001	21.10	2/112	<0.001	0.274
	SWM_BE	1.47	0.445	3.30	0.001				

PMRI1: psychomotor retardation index 1

PMRSI2: psychomotor retardation index 2

MOT_ML: motor screening taks, mean latency

RVP_ML: rapid visual information process test, median latency

OTS: One touch stockings of Cambridge, probability solved on first choice

SWM_BE: Spatial working memory, between errors

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Table 3. Results of neural network models with schizophrenia (SCZ) and major neuro-cognitive disorder (MNP) as output variables and psychomotor retardation, formal thought disorders, negative symptoms, psychosis and excitation as input variables

Network information		**Model 1: SCZ vesus controls**	**Model 2: MNP versus rest**
Number units		6	6
Hidden layers	Number hidden layers	2	2
	Number units in layer 1	4	4
	Number units in layer 2	3	3
	Activation function	Hyperbolic tangent	Hyperbolic tangent
Output layer	Activation function	Identity	Identity
Training	Sum of squares error	2.138	3.324
	Percent correct predictions	93.3% HC: 94.1% SCZ: 93.0%	97.3% HC/SNP: 98.0% MNP: 95.5%
Testing	Sum of squares error	0.918	1.112
	Percent correct predictions	96.9% HC: 100% SCZ: 94.1%	94.4% HC/SNP: 100.0% MNP: 80%
Hold-out	Percent correct predictions	96.3% HC: 100% SCZ: 94.7%	100.0% HC/SNP: 100.0% MNP: 100.0%

Rest: patients with simple neuro-cognitive psychosis and healthy controls (HC)

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Table 4. Characteriztics of schizophrenia (SCZ) patients with and without severe psychomotor retardation (PMR) and healthy controls (HC).

Variables	**HC** [A]	**PMRI1 < median** [B]	**PMRI1 > median** [C]	**F/ΨX²**	**df**	**P**
PMRI1	-0.772 (0.142) [B,C]	-0.436 (0.397) [A,C]	1.197 (0.762) [A,B]	174.73	2/116	<0.001
PMRI2	-0.731 (0.213) [B,C]	-0.352 (0.400) [A,C]	1.075 (0.983) [A,B]	92.28	2/116	<0.001
MOT_ML (ms)	947 (438) [C]	1119 (445) [C]	1407 (783) [A,B]	9.25	2/116	<0.001
MOT_ME (pixel units)	11.04 (4.13)	11.40 (2.79)	9.63 (3.15)	2.99	2/116	0.054
MOT_TC (correct answers)	9.8 (0.9)	9.8 (0.8)	9.5 (1.3)	1.08	2/116	0.343
Age (years)	37.4 (12.8)	41.1 (11.7)	40.9 (10.5)	1.27	2/116	0.285
Sex (M/F)	10 / 30 [B]	25 / 14 [A]	18 / 22	12.23	1	0.002
Education (years_	14.3 (4.9) [C]	12.8 (3.6)	11.8 (4.7) [A]	3.25	2/116	0.042
Single / married / separated	23 / 14 / 3	28 / 4 / 5	31 / 7 / 2	Ψ = 0.27	-	0.067
Body mass index (kg/m^2)	24.0 (4.3)	25.8 (6.0)	23.4 (3.7)	2.71	2/111	0.071
Smoking (No/Yes)	38 / 2	36 / 3	28 / 2	0.34	1	0.842
Duration psychosis (years)	-	16.6 (9.5)	12.5 (10.9)	2.95	1/71	0.090
First episode / multiple	-	5 / 19	5 / 28	0.31	1/72	0.578
HC / Non-Deficit / Deficit SCZ	-	29 / 11	10 / 29	17.35	1	<0.001
HC / SNP / MNP	-	30 / 13	9 / 26	14.98	1	<0.001
SDS score	0.0 (0.0) [B,C]	3.44 (3.27) [A,C]	10.13 (5.96) [A,B]	68.42	2/116	<0.001
Total SANS score	0.5 (1.74) [B,C]	22.3 (16.1) [A,C]	47.5 (23.6) [A,B]	80.88	2/116	<0.001
Psychosis (z score)	-0.820 (0.0) [B,C]	-0.041 (0.869) [A,C]	0.860 (0.917) [A,B]	53.35	2/116	<0.001
Hostility (z score)	-0.595 (0.0) [B,C]	0.213 (1.270) [A]	0.386 (0.945) [A]	13.21	2/116	<0.001
Excitation (z score)	-0.809 (0.0) [B,C]	-0.105 (0.803) [A,C]	0.883 (0.954) [A,B]	55.86	2/116	<0.001
Mannerism (z score)	-0.737 (0.0) [B,C]	0.096 (0.993) [A,C]	0.643 (1.037) [A,B]	28.19	2/116	<0.001
HDRS score	0.6 (2.0) [B,C]	4.0 (2.9) [A,C]	10.6 (5.8) [A,B]	67.15	2/116	<0.001

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PMRI1: psychomotor retardation index 1

PMRSI2: psychomotor retardation index 2

MOT_ML: motor screening taks, mean latency; MOT_ME: MOT, mean error; MOT_TC: MOT total correct responses

HC / SNP / MNP: healthy controls / simple neuro-cognitive psychosis / major neuro-cognitive psychosis

SDS: Schedule for the Deficit Syndrome

SANS: Scale for the Assessments of Negative Symptoms

HDRS: Hamilton Depression Rating Scale

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Table 5. Neurocognitive tests and biomarkers in schizophrenia patients with and without severe psychomotor retardation (PMR) and healthy controls.

Variables	**Controls** [A]	**PMRI1 < median** [B]	**PMRI1 > median** [C]	**F/ΨX²**	**Df**	**P**
Mini Mental State Examination	28.3 (2.3) [B,C]	26.8 (3.0) [A,C]	24.8 (4.0) [A,B]	12.94	2/116	<0.001
Verbal Fluency Test	26.6 (6.3) [B,C]	20.2 (6.2) [A,C]	16.8 (6.1) [A,B]	25.93	2/116	<0.001
Word List Memory	22.1 (4.4) [B,C]	18.9 (4.0) [A,C]	14.6 (5.5) [A,B]	25.93	2/116	<0.001
Word List Recall	8.1 (1.8) [B,C]	7.1 (1.7) [A,C]	5.3 (2.3) [A,B]	20.46	2/116	0.032
Formal thought disorder (z score)	-0.761 (0.0) [B,C]	0.084 (1.078) [A,C]	0.679 (0.906) [A,B]	31.92	2/116	<0.001
RVP_A′	0.950 (0.159) [B,C]	0.943 (0.063) [A,C]	0.856 (0.223) [A,B]	4.09	2/116	0.019
RVP_ML	373.9 (105.7) [B,C]	475.8 (130.3) [A]	526.4 (221.5) [A]	9.34	2/116	<0.001
SWM_STR	34.4 (5.0) [B,C]	39.7 (5.8) [A]	38.8 (8.0) [A]	7.80	2/116	0.001
SWM_BE	27.9 (22.5) [B,C]	54.6 (22.3) [A]	57.1 (23.6) [A]	19.94	2/116	<0.001
OTS_PSOFC	8.8 (4.4) [B,C]	5.4 (3.2) [A]	4.6 (2.9) [A]	15.70	2/116	<0.001
Executive Pricipal component	-0.724 (0.961) [B,C]	0.309 (0.888) [A]	0.435 (0.740) [A]	21.59	2/116	<0.001
IgM MDA	0.275 (0.821) [C]	0.078 (1.155) [C]	-0.349 (0.923) [A,B]	4.16	2/114	0.018
Immune activation index (z score)	-0.674 (0.857) [B,C]	0.368 (0.773) [A]	0.339 (0.996) [A]	18.07	2/114	<0.001
CCL-11 (pg/mL)	129.6 (54.1) [B,C]	208.6 (78.5) [A]	216.5 (118.9) [A]	18.88	2/116	<0.001
IgA NOX/PRO (z score)	-0.695 (0.654) [B,C]	0.320 (1.059) [A]	0.331 (0.840) [A]	18.48	2/116	<0.001
IgM NO-Cysteinyl (z score)	0.021 (0.699)	-0.033 (1.267)	-0.001 (0.980)	0.03	2/116	0.972

PMRI1: psychomotor retardation index 1

RVP_A and RVP_ML: rapid visual information process test, prime A′ and median latency

SWM_STR and SWM_BE: Spatial working memory, strategy and between errors

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OTS_PSOFC: One touch stockings of Cambridge, probability solved on first choice

Executive Lalent Vector, extracted from SWM_STR, SWM_BE and OTS_PSOFC

IgM MDA: natural IgM directed to malondialdehyde

Immune activation index: computed as z score interleukin-10 (zIL-10) + z Macrophage inflammatory protein 1 + z soluble interleukin-1 receptor antagonist

IgA NOX/PRO: ratio of noxious versus more protective tryptophan catabolites (TRYCATs)

IgM NO-Cysteinyl: IgM directed to nitroso-cysteinyl

Table 6. Results of multiple regression analysis with the psychomotor retardation (PMR) and motor screening task (MOT) scores as dependent variables and biomarkers as explanatory variables.

Dependent Variable	Explanatory Variables	B	SE	t	p	F model	df	P	Partial Eta squared
PMRI1	IgM MDA	-0.548	0.095	-5.75	<0.001	18.45	4/112	<0.001	0.397
	IgM NO-Cysteinyl	0.413	0.093	4.42	<0.001				
	CCL-11	0.298	0.080	3.74	<0.001				
	IgA NOX/PRO	0.190	0.078	2.45	0.016				
PMRI2	IgM MDA	-0.579	0.095	-6.12	<0.001	21.48	4/112	<0.001	0.434
	IgM NO-Cysteinyl	0.355	0.092	3.85	<0.001				
	CCL-11	0.295	0.075	3.93	<0.001				
	IgA NOX/PRO	0.206	0.076	2.72	0.001				
MOT_ML	IgM MDA	-0.222	0.088	-2.51	0.014	7.99	3/114	<0.001	0.170
	Immune activation	0.194	0.091	2.14	0.035				
	CCL-11	0.189	0.093	2.04	0.043				

PMRI1: psychomotor retardation index 1

PMRSI2: psychomotor retardation index 2

MOT_ML: motor screening task, median latency

IgM MDA: IgM directed to malondialdehyde

IgM NO-Cysteinyl: IgM directed to nitroso-cysteinyl

Immune activation index: computed as z value interleukin-10 + z Macrophage Inflammatory Protein + z soluble IL-1 receptor antagonist

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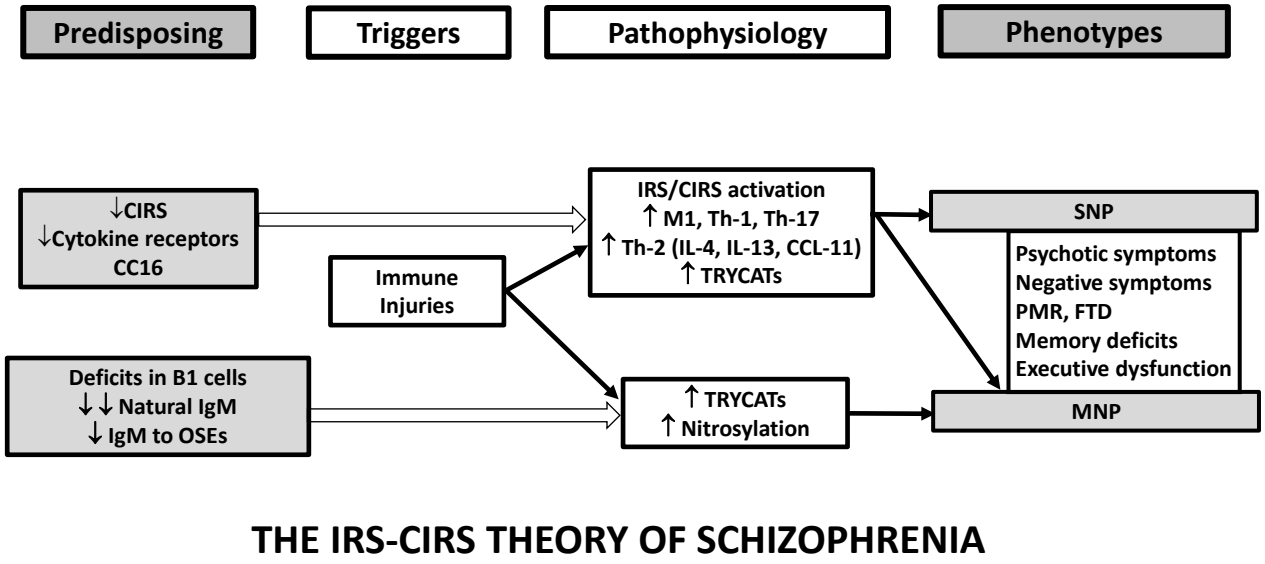

Figure 1. The author's new schizophrenia theory. Trigger factors including infections may activate the immune response system (IRS) and the compensatory immune-regulatory system (CIRS) (Roomruangwong et al., 2018b). Nevertheless, when there are pre-existing deficits in the CIRS, including lowered levels of Clara Cell protein (CC16) and soluble cytokine receptor levels, the immune-regulatory CIRS functions may be insufficient thereby increasing risk towards detrimental effects of an activated IRS (Roomruangwong et al., 2018b). Many IRS products have cytotoxic and neurotoxic effects, which may cause neuroprogression and, consequently, schizophrenia phenomenology (Roomruangwong et al., 2018b). These products include M1 macrophagic-related cytokines; T helper (Th)-1 cytokines

and trytptophan catabolites (TRYCATs) and Th-2 products such as interleukin (IL)-4, IL-13 and eotaxin (CCL-11) (Kanchanatawan et al., 2018a; Sirivichayakul et al., 2018; 2019; Roomruanwong et al., 2018b). However, pre-existing deficits in B1 cell functions including lower natural IgM (Maes et al., 2018), are accompanied by increased neurotoxic effects of TRYCATs and nitrosylated proteins (Maes et al., 2018). All those factors together may contribute to negative symptoms, psychosis, excitation and neurocognitive deficits thereby shaping major (MNP) and simple neuro-cognitive psychosis (SNP).

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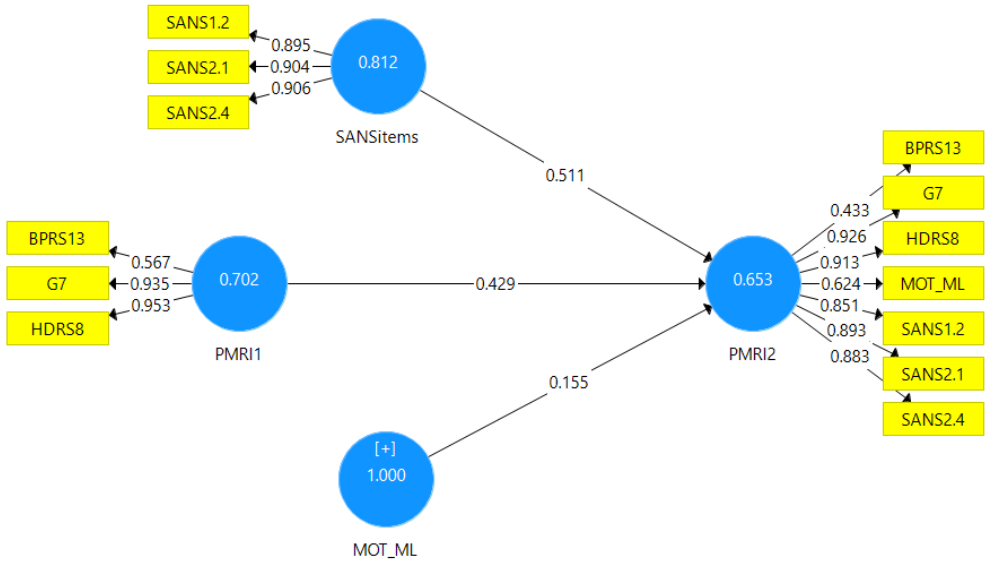

Figure 2. Construction of two psychomotor retardation indices, namely PMRI1 and PMRI2. PMRI1 was constructed by entering 3 items, namely HDRS8, PANSSG7 and BPRS13, as indicators, while the second index was constructed using these three indicators coupled with MOT_PL, SANS1.2, SANS2.1 and zSANS2.4 (see Table 1 for more explanation). Shown are path coefficients with exact p-values and p-values for the outer model.

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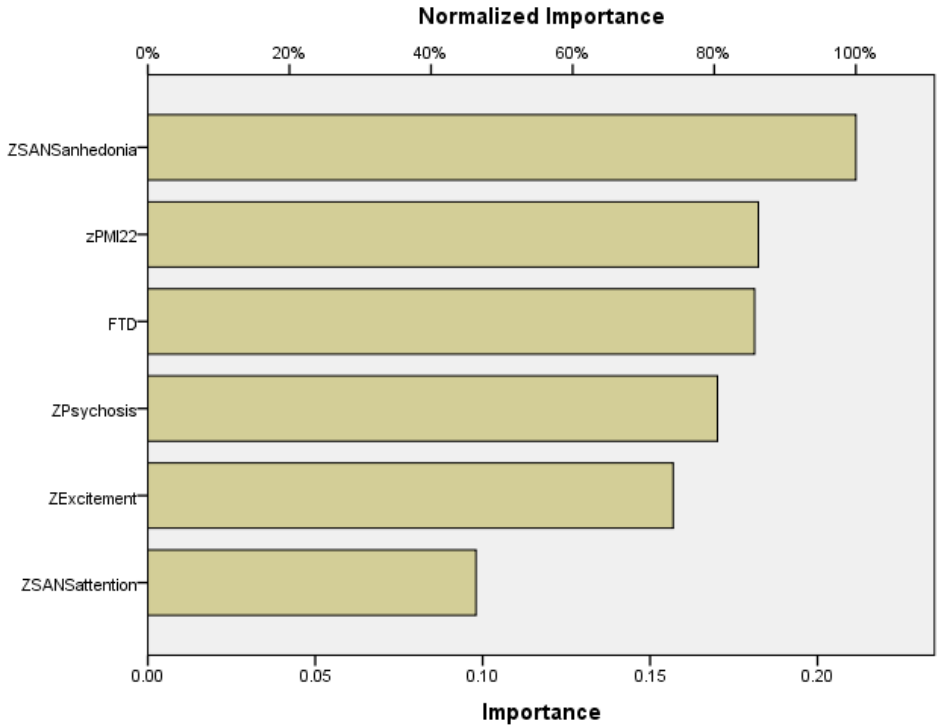

Figure 3. Results of Neural Network analysis, namely the importance chart showing the significance of the input variables discriminating schizophrenia from controls. Anhedonia (zSANSanhedonia), psychomotor index (PMRI2), formal thought disorders (FTD), psychosis and excitement are the most important determinants of the predictive power of the model, while attention (ZSANSattention) follows at a distance.

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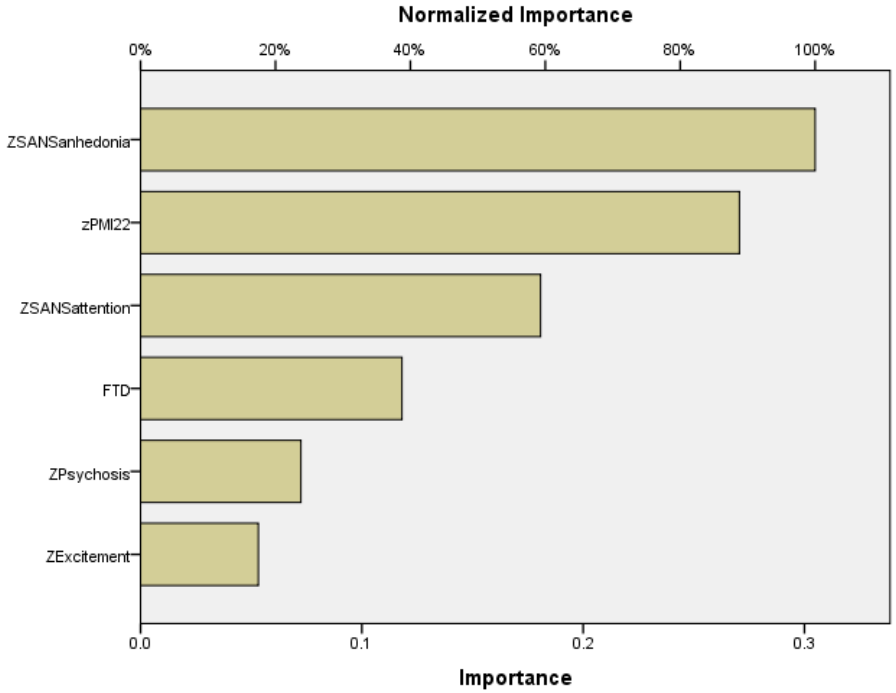

Figure 4. Results of Neural Network analysis, namely the importance chart showing the significance of the input variables discriminating major neuro-cognitive psychosis (MNP) from simple neuro-cognitive psychosis and controls. This importance chart is dominated by anhedonia (ZSANSanhedonia) and psychomotor slowing (zPMI22), while attention (ZSANSattention) and formal thought disorders (FTD) are relevant determinants, which follow at a distance.

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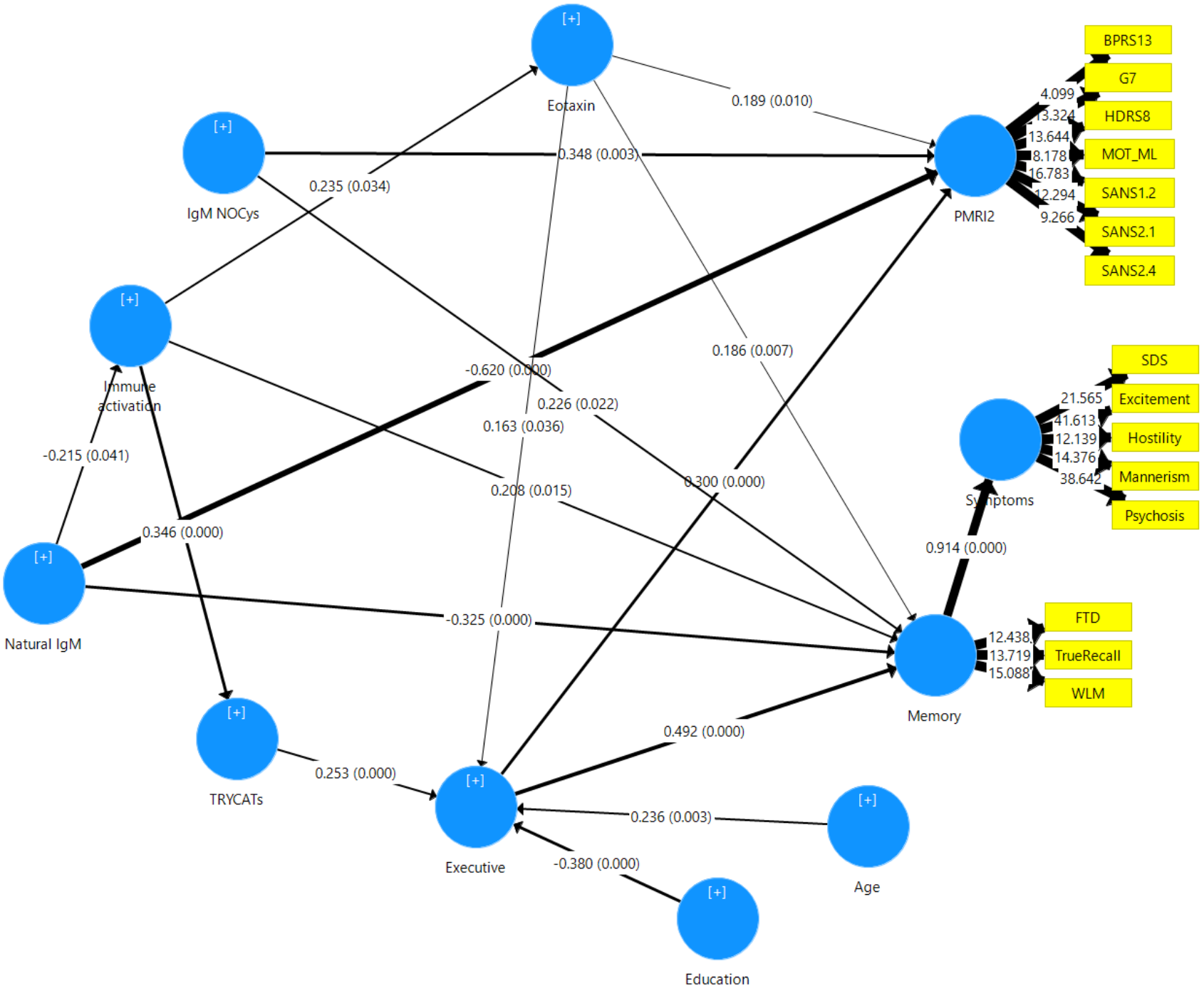

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Figure 5. Results of Partial Least Squares analysis with psychomotor retardation and schizophrenia symptoms as output variables. This path model examines the causal links between the biomarkers (explanatory variables) and executive functions (entered as an indicator variable), which together predict psychomotor retardation latent vector (LV, PMRI2), extracted from the 7 indicators described in Table 1, memory functions, entered as a LV extracted from True Recall, World List Memory (WLM) and Formal Thought Disorders (FTD), and a symptom LV, with 5 indicators, namely the SDS total score and psychosis, excitement, hostility and mannerism. The biomarkers are eotaxin (CCL-11), IgM to nitroso-cysteinyl (NO-Cys), immune activation (entered as a z composite score based on measurements of plasma cytokines/receptor levels), IgM antibodies to malondialdehyde (natural IgM), and tryptophan catabolites (TRYCATs). Age and years of education were entered as additional explanatory variables predicting executive functions. Shown are path coefficients with exact p-values and t-values for the outer model.

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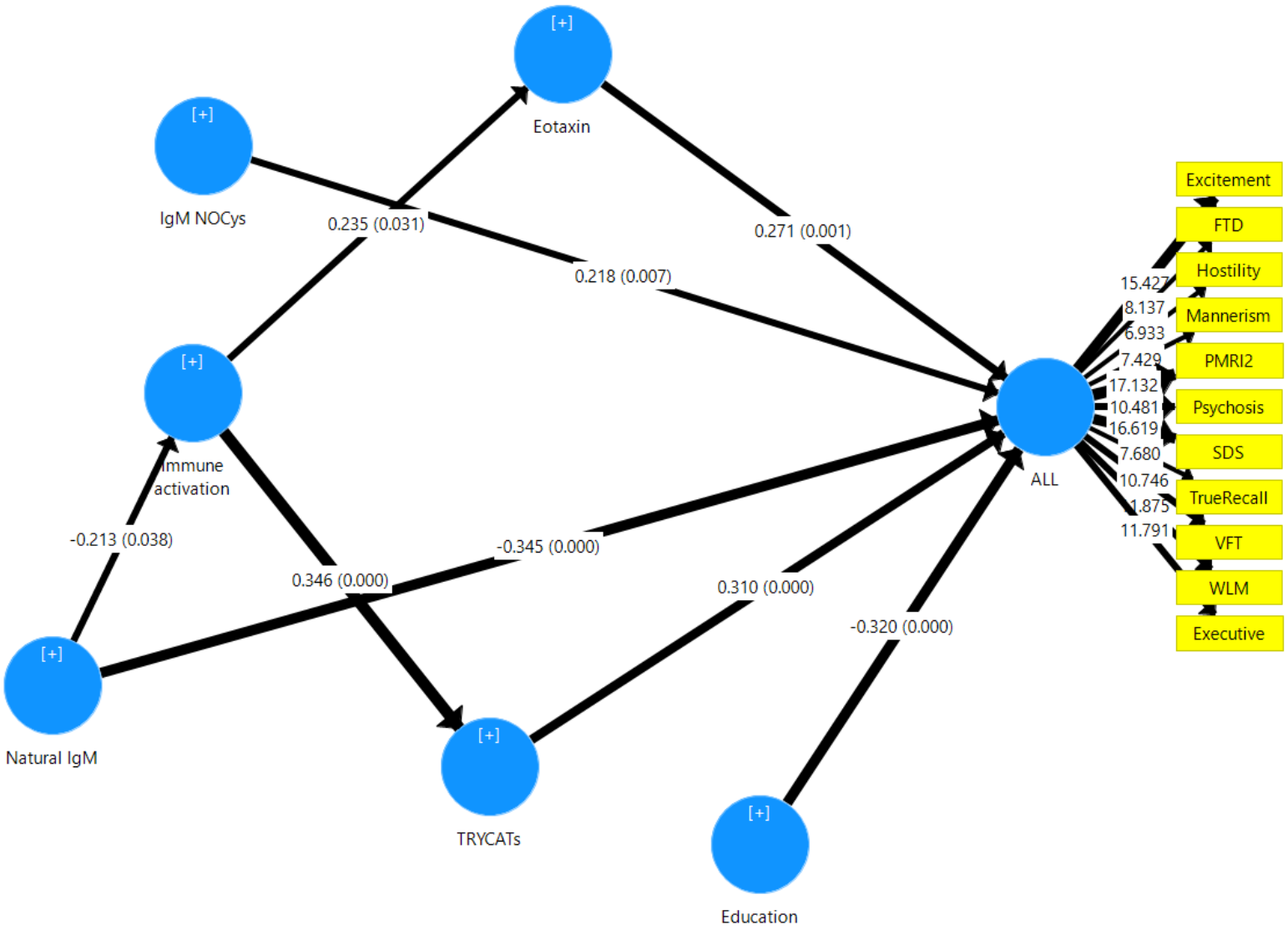

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Figure 6. Results of Partial Least Squares analysis with the psychomotor retardation index PMRI2 combined with negative symptoms (SDS), executive functions, True Recall, Verbal Fluency Test (VFT), World List Memory (WLM), Formal Thought Disorders (FTD), psychosis, excitement, hostility, mannerism as indicators of a general psychopathology LV. Input variables are the biomarkers (see Figure 5 for explanation). Shown are path coefficients with exact p-values and t-values for the outer model.