



Inflammatory biomarkers in psychosis and clinical high risk populations

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ABSTRACT

Background: Immunological, nutritional, and microbial factors have been implicated in the pathophysiology of schizophrenia, but the interrelationship among measures is understudied. In particular, an increase in the levels of the pro-inflammatory cytokine interleukin-6 (IL-6) is associated with all phases of the illness, and may be associated with other inflammatory markers.

Vitamin D is a modulator of the immune system, and LPS antibodies are an indirect measure of gut barrier function. In this study we investigated potential contributing inflammatory mechanisms for IL-6 elevation.

Methods: We compared the levels of vitamin D, C-reactive protein (CRP), antibodies to lipopolysaccharide (LPS), and IL-6 in children, adolescents and young adults with psychosis ($n = 47$), individuals at clinical high risk for psychosis ($n = 17$) and unaffected comparison controls ($n = 33$). Participants were diagnosed by a psychiatrist, using a structured interview, the MINI-Neuropsychiatric Interview. 25(OH)D was measured in serum using chemiluminescent micro particle immunoassay, and anti-LPS antibodies, CRP and IL-6 levels were measured by ELISA.

Results: IL-6 and C-reactive protein levels were significantly elevated in the psychosis group relative to the unaffected control subjects. In the psychosis group, levels of IL-6 correlated positively with IgA anti-LPS antibodies and negatively correlated with vitamin D.

Conclusions: Our findings show a significant correlation between IL-6, anti-LPS antibodies and vitamin D deficiency in psychosis, suggesting the existence of multiple potential pathways related to IL-6 elevation in psychosis, and therefore multiple potential strategies for risk mitigation. Collectively these findings support hypotheses regarding interrelated inflammatory contributions to the pathophysiology of psychosis.

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1. Introduction

Schizophrenia affects approximately 1% of the population, with a pathophysiology that is largely elusive. Inflammatory models of schizophrenia abound, and consistent literature showing increased plasma levels of certain interleukin (IL) cytokines, especially IL-6, in all ages and phases of psychosis (Stojanovic et al., 2014). The etiology for elevated IL-6 in psychosis remains to be determined. In immune-mediated conditions such as multiple sclerosis and Crohn's disease, high IL-6 levels have been linked to nutritional factors such as vitamin D deficiency, and have been shown to influence clinical status (Reich et al., 2016). Vitamin D has been found to be deficient in patients with schizophrenia (Graham et al., 2015) and linked to reduced hippocampal volume (Shivakumar et al., 2015). Vitamin D is a modulator of the

immune system, and controls the development of the largest immune organ, gut-associated lymphoid tissue (GALT) (Weiss, 2011). The gut microbiome has emerged as a central link between inflammation and neuropsychiatric disease (Sandhu et al., 2017). LPS antibodies may also serve as an indirect measurement of LPS leakage into the circulation, reflecting a defect in gut barrier function (Barclay, 1995).

Our study aimed at investigating the potential causes of IL-6 elevation in those clinically high risk (CHR) for psychosis, and in adolescents and young adults with psychosis, using a cross-sectional approach across a range of potential etiological factors. Our primary hypothesis was that we would find a significant difference in IL-6 levels between psychosis participants and controls, and would correlate with inflammatory biomarkers such as vitamin D and LPS antibodies.

2. Subjects and methods

This study was approved by the New York State Psychiatric Institute Institutional Review Board. All participants (age 8–35) signed informed

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consent; in the case of those under age 18, assent was signed and parents also signed consent. Participants were classified into one of three groups: 1) Children, adolescents and young adults with psychosis ($n = 47$), 2) clinical high risk (CHR) for psychosis ($n = 17$) and 3) unaffected by psychosis controls ($n = 33$).

2.1. Participants

Inclusion criteria consisted of a diagnosis of psychosis between the ages of 8 and 35. In the psychosis group, 4 were children or adolescents with psychosis (ages 12–17) and 43 were young adults with psychosis (ages 18–35). A psychiatrist administered the MINI Neuropsychiatric structured diagnostic interview, and eligible participants with psychosis had a positive diagnosis of either psychosis (current or lifetime), or mood disorder with psychotic symptoms. CHR participants, ages 14–30 were recruited solely through the prodromal clinic, the Center for Prevention and Evaluation (COPE). Unaffected comparison controls were recruited through Columbia's clinical research website.

2.2. Blood sampling and laboratory analysis

Serum and plasma was collected on all participants and stored at -80°C until use for assays. Vitamin D3 25OH was immediately measured in the serum using a quantitative chemiluminescent immunoassay. Serum IL-6, CRP and plasma IgG, IgA and IgM LPS antibodies were all measured by a high sensitivity enzyme-linked immunosorbent assay (ELISA) kits, according to manufacturer protocols.

2.3. Statistical analysis

Kruskal-Wallis tests were used to compare demographics and biomarkers between the three groups (Table 2). To compare IL-6 and CRP levels between groups, multinomial logistic model was fitted with group as the outcome controlling for LPS IgA, vitamin D, season of sampling and demographic variables including age, gender and BMI.

The bivariate association between IL-6, vitamin D, and LPS antibodies in each group was examined using Spearman's correlation analysis. Partial correlation was examined using multiple linear regression with IL-6 as the outcome and vitamin D and LPS IgA as independent variables. In the regressions, log transformation was applied to IL-6, CRP and anti-LPS antibodies. All statistical tests were two-sided and were considered significant at $p \leq 0.05$. SAS 9.4 was used for all comparisons.

3. Results

Demographic information for all groups is summarized in Table 1. There was no difference across groups in age, gender, or BMI. The levels of vitamin D and LPS antibodies were not statistically different between groups (Table 2).

Table 1 Demographics by group.

Variable	Group			Test statistic	p value
	Psych (N = 47)	CHR (N = 17)	Control (N = 33)		
Age				3.54	0.17
N	47	17	33		
Mean (SD)	24.30 (5.91)	22.76 (3.68)	25.27 (4.75)		
BMI				4.56	0.10
N	47	17	33		
Mean (SD)	27.80 (7.88)	23.87 (4.02)	24.84 (6.26)		
Male, N (%)	30 (63.83)	11 (64.71)	14 (42.42)	3.6	0.17

Table 2 Biomarkers by group.

Variable	Group			Test statistic	p value
	Psych (N = 47)	Early (N = 17)	Control (N = 33)		
Sampled at seasons 1 and 2, N (%)	28 (59.57%)	12 (70.59%)	18 (54.55%)	1.20	0.55
Vitamin D				0.59	0.74
N	47	17	30		
Mean-SD	28.5 (13.8)	25.18 (10.61)	28.05 (12.23)		
IgA				0.05	0.98
N	42	17	31		
Mean-SD	0.05 (0.05)	0.06 (0.08)	0.05 (0.05)		
IgG				0.76	0.69
N	42	17	31		
Mean-SD	0.11 (0.07)	0.09 (0.06)	0.12 (0.12)		
IgM				1.83	0.40
N	42	17	31		
Mean-SD	0.12 (0.09)	0.18 (0.16)	0.15 (0.11)		
CRP				7.08	0.03
N	47	17	32		
Mean-SD	4376 (3797.04)	2654 (2729)	2433 (2907)		

3.1. Between group comparisons

The IL-6 level in psychosis group was significantly higher than that in the controls, (Wald $\chi^2 = 5.11, p = .024$) (Fig. 3), controlling for LPS IgA, vitamin D, season of sampling, and demographics (Fig. 1). CRP was also increased in the psychosis group compared to the controls (Wald $\chi^2 = 5.11, p = .024$). By contrast, there were no significant differences in IL-6 (Wald $\chi^2 = 1.73, p = .19$) or CRP (Wald $\chi^2 = 1.25, p = .26$) between the CHR group and controls.

Without controlling for demographics and inflammatory markers, no significant differences in IL-6 and CRP were observed between groups.

3.2. Within group correlations

3.2.1. IL-6 and vitamin D

Elevated IL-6 and low vitamin D were significantly correlated across all three groups ($r = -0.40, p < .0001$) and remained significant even when controlling for group status ($F_{1,88} = 9.13, p = .003$). In the

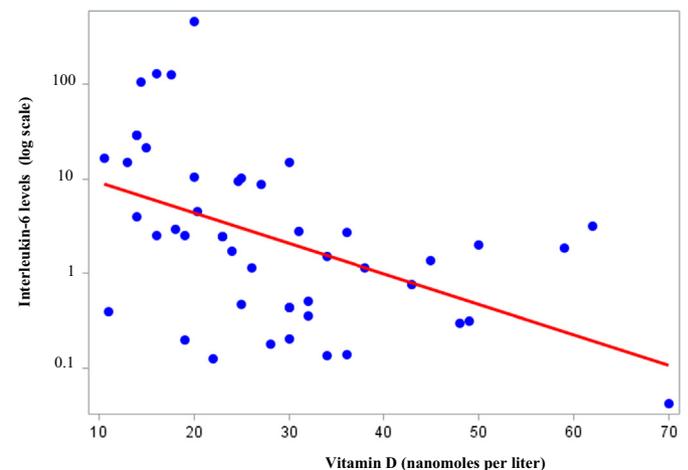


Fig. 1. Scatter plot with regression line of LogIL6 and vitamin D in psychosis group.

psychosis group, IL-6 had a highly significant negative correlation with vitamin D (Table 3, $r = -0.49, p < .0001$). In the CHR group, the relationship between IL-6 and vitamin D was again negative but was not statistically significant ($r = -0.21, p = .4$). In the control group, the relationship was again significant ($r = -0.37, p = .05$).

3.2.2. IL-6 and LPS antibodies

IL-6 and anti-LPS IgA significantly correlated across all three groups, and the correlation remained significant following control for group status ($F_{1,84} = 4.785, p = .031$). In the psychosis group, there was a positive correlation with IL-6 and anti-LPS IgA ($r = 0.36, p = .02$). By contrast, there were no significant relationships between IL-6 and LPS antibodies in either the CHR ($r = 0.13, p = .6$) or control ($r = 0.15, p = .4$) groups.

3.2.3. IL-6 and BMI

IL-6 and BMI are significantly correlated across all three groups ($r = 0.55, p < .001$), but the relationship was significant at trend level when controlled for group status ($F_{1,91} = 3.794, p = .055$). In the psychosis group, IL-6 and BMI were positively associated ($r = 0.70, p < .0001$), as well as the control group ($r = 0.41, p = .02$) but not in the CHR group ($r = -0.18, p = .5$).

3.3. Multiple regression analysis

When a MANCOVA approach was used controlling for group, Vitamin D ($F = 13.7, df = 1,76, p < .000$) and BMI ($F = 9.71, df = 1,76, p < .001$) emerged as independent predictors, whereas the contribution of IgA was significant at trend level only ($F = 3.67, df = 1,76, p = .06$). In within-group multiple regression for chronic patients, the combination of BMI and Vitamin D levels accounted for ~50% of the variance in IL-6 levels ($R^2 = 0.58, p = .001$), with significant independent contributions for each measures (BMI: partial $r = -.68, p < .001$; Vitamin D: partial $r = -.51, p = .001$).

Table 3
Pearson correlation between IL-6, vitamin D and LPS antibodies.

	IL6	CRP	Vit D	IgA	IgG	IgM
Overall						
CRP	0.38**					
Vit D	-0.40**	-0.11				
IgA	0.22*	0.06	-0.12			
IgG	0.19	-0.03	-0.14	0.38**		
IgM	-0.01	-0.04	-0.01	0.40**	0.20*	
BMI	0.55**	0.44**	-0.20	0.12	0.14	-0.26*
Psychosis						
CRP	0.48**					
Vit D	-0.48**	-0.20				
IgA	0.36*	0.31	-0.33*			
IgG	0.22	-0.03	-0.16	0.41**		
IgM	0.05	0.17	-0.12	-0.02	-0.02	
BMI	0.70**	0.44*	-0.21	0.28	0.34*	-0.18
CHR						
CRP	-0.08					
Vit D	-0.19	-0.35				
IgA	0.16	-0.32	0.14			
IgG	0.25	-0.11	-0.11	0.78**		
IgM	0.10	-0.41	0.36	0.71**	0.44	
BMI	-0.18	0.49	-0.17	-0.15	-0.13	-0.45
Unaffected controls						
CRP	0.27					
Vit D	-0.40*	0.08				
IgA	0.15	-0.12	-0.07			
IgG	0.20	0.01	-0.17	0.28		
IgM	-0.04	0.02	-0.09	0.42*	0.32	
BMI	0.41*	0.29	-0.31	0.14	0.01	-0.24

* $p < .05$.

** $p < .01$.

4. Discussion

To our knowledge, this is the first study investigating IL-6 levels, antibodies to LPS, and vitamin D in a population of individuals with psychosis and CHR. Consistent with prior literature, our data show that IL-6 and CRP are elevated among participants with psychosis, supporting a possible role of inflammation in the pathophysiology of psychosis. The differences in IL-6 and CRP were observed only after controlling for potentially confounding factors. We interpret this as indicating greater IL6 levels in schizophrenia patients even after controlling for demographic, metabolic and nutritional factors that are known to affect IL-6 expression.

A novel finding from this study was the highly significant negative correlation between vitamin D and IL-6 (Table 2, $r = -0.56, p < .0001$) in the psychosis group, to a greater degree than in controls (Table 2, $r = -0.37, p = .05$). While this has been reported in a study of suicidal patients (Grudet et al., 2014) this has not yet been studied in the psychosis population. Mechanistically we think this converges on the toll-like receptor 4 (TLR4), as in-vitro studies show that vitamin D deficiency can also lead to increased IL-6, whereby vitamin D inhibited pro-inflammatory cytokine production through MAPK phosphatase-1 (Zhang et al., 2012). Our study suggests that given its relationship with IL-6, vitamin D may play a mediating role in psychosis risk. There is a paucity of clinical trials investigating the efficacy of vitamin D supplementation in those with neuropsychiatric disease, and especially those with psychosis; an 8-week vitamin D supplementation trial improved symptoms in bipolar youth with mania, and also increased levels of GABA in the anterior cingulate cortex (ACC) using MRS (Sikoglu et al., 2015). In schizophrenia, one open label 8-week vitamin D supplementation trial investigated the effect of vitamin D on metabolic markers, and showed a significant decrease in cholesterol in participants whose vitamin D level was 30 or greater (Thakurathi et al., 2013). Another study gave vitamin D supplementation to those with chronic schizophrenia on clozapine, and found a trend towards better cognition (Krivoy et al., 2017). Surprisingly, we did not find a difference between vitamin D levels among groups, largely due to the fact that a significant number of controls were found to have vitamin D insufficiency or deficiency (see Table 2).

In schizophrenia, studies have examined serological markers of bacterial translocation, sCD14 and LPS-binding protein; sCD14 seropositivity conferred a 3.1-fold increased odds of association with schizophrenia (Severance et al., 2013). Another novel and important finding is the correlation between IL-6 and LPS IgA antibodies found in the psychosis group (Table 3, Fig. 2), which suggests the potential role of bacterial translocation in the disease process of psychosis. IgA antibodies reflect a mucosal immune response, (Brandtzaeg, 2007) and vitamin D and

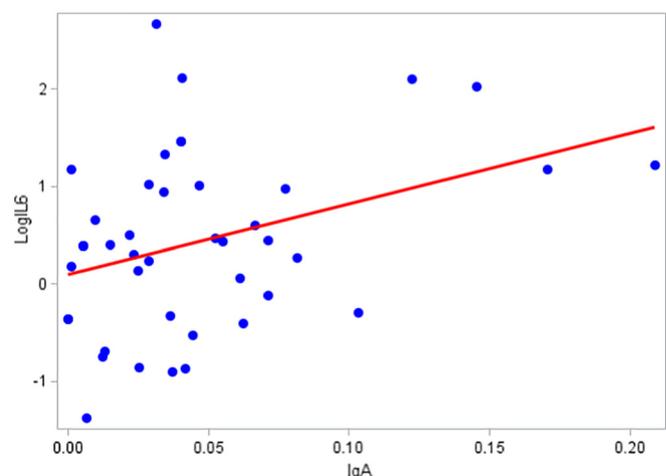


Fig. 2. Scatter plot with regression line of Log10IL6 and IgA in psychosis group.

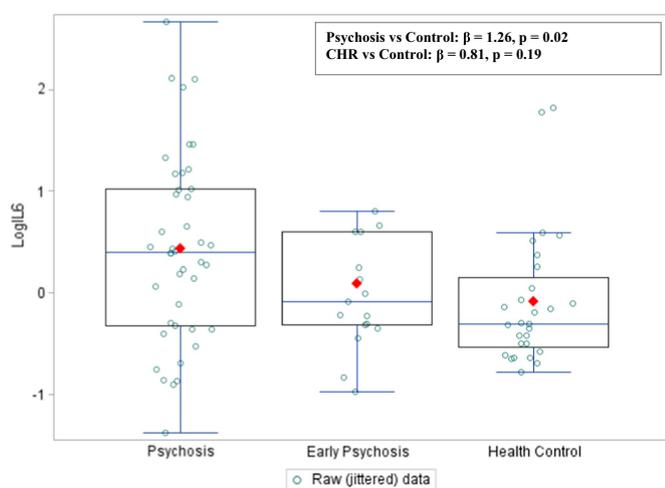


Fig. 3. Boxplots of Interleukin-6 levels by group.

vitamin A are critical regulators of mucosal immune function (Kunisawa and Kiyono, 2013). Our findings in the psychosis group only support this mechanism, as we see an inverse relationship between LPS IgA antibodies and vitamin D (Table 3), suggesting that these alterations in the gut microbiome, in concert with vitamin D deficiency, may contribute to elevated IL-6 levels in psychosis patients to a greater degree than in controls.

Our data shows a significant relationship between IL-6 and BMI (Table 3) which is well-established in the literature (Khaodhiar et al., 2004). The relationship is most pronounced in the psychosis group (Table 2) underscoring the importance of careful metabolic management in those with psychosis.

Our secondary aim was to compare relationships among CHR subjects to those observed in established psychosis. We did not find significant group differences in inflammatory biomarkers between those with psychosis and CHR.

Our results showing elevated IL-6 levels, LPS IgA antibodies and low vitamin D in the psychosis group may biologically converge on the toll-like receptor 4. The TLR4 has recently emerged as playing a role in psychiatric diseases such as schizophrenia, and also autoimmune disorders and CNS disorders (Garcia Bueno et al., 2016). The binding of LPS to the TLR4 receptor results in the production of a variety of pro-inflammatory cytokines, such as IL-6 (Cai et al., 2015). In mouse models Vitamin D deficiency can also cause disruption of the gut barrier (Asa et al., 2014). Cytokines such as IL-6 and enteric microbes containing LPS can regulate tight junctions and intestinal barrier integrity (Turner, 2009). We hypothesize that in psychosis patients, chronically low vitamin D may contribute to intestinal barrier permeability and act in concert with LPS to cause an increased inflammatory state through TLR4, leading to increased cytokine production.

5. Conclusions

Our data suggest that biomarkers such IgA antibodies to LPS, increased levels of IL-6, and vitamin D deficiency collectively may contribute to the inflammatory state in patients with psychosis and may also contribute to gut and blood brain barrier dysfunction. Clinical trials of vitamin D may prove to be a simple intervention that could improve clinical symptoms and may play a significant role in prevention as well.

Conflicts of interest

All authors declare no conflicts of interest related to this work.

Contributors

Dr. Shannon Delaney contributed to study design, data collection, analysis, interpretation of data and writing of the manuscript. Dr. Daniel Javitt contributed to data analysis

and statistics, interpretation of data, figures and editing of the manuscript. Dr. Brian Fallon contributed to study design, interpretation of data and editing of manuscript. Dr. Robert Yolken, Dr. Armin Alaedini and Alyssa Indart contributed to data collection and analysis. Dr. Yuanjia Wang and Tianshu Feng contributed to statistics. All authors have approved the final manuscript.

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References

- Asa, A., Vong, L., Pinnell, L.J., Avitzur, N., Johnson-Henry, K.C., Sherman, P.M., 2014. Vitamin D deficiency promotes epithelial barrier dysfunction and intestinal inflammation. *J. Infect. Dis.* 210, 1296–1305.
- Barclay, G.R., 1995. Endogenous endotoxin-core antibody (EndoCAB) as a marker of endotoxin exposure and a prognostic indicator: a review. *Prog. Clin. Biol. Res.* 392, 263–272 (cited 2017 Jan 28). (Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8524931>).
- Brandtzaeg, P., 2007. Induction of secretory immunity and memory at mucosal surfaces. *Vaccine* 25, 5467–5484.
- Cai, B., Wang, M., Zhu, X., Xu, J., Zheng, W., Zhang, Y., Zheng, F., Feng, Z., Zhu, J., 2015. The Fab fragment of a humanized anti-toll like receptor 4 (TLR4) monoclonal antibody reduces the lipopolysaccharide response via TLR4 in mouse macrophage. *Int. J. Mol. Sci.* 16, 25502–25515.
- Garcia Bueno, B., Caso, J.R., Madrigal, J.L., Leza, J.C., 2016. Innate immune receptor Toll-like receptor 4 signalling in neuropsychiatric diseases. *Neurosci. Biobehav. Rev.* 64, 134–147.
- Graham, K.A., Keefe, R.S., Lieberman, J.A., Calikoglu, A.S., Lansing, K.M., Perkins, D.O., 2015. Relationship of low vitamin D status with positive, negative and cognitive symptom domains in people with first-episode schizophrenia. *Early Interv. Psychiatry* 9, 397–405.
- Grudet, C., Malm, J., Westrin, A., Brundin, L., 2014. Suicidal patients are deficient in vitamin D, associated with a pro-inflammatory status in the blood. *Psychoneuroendocrinology* 50, 210–219.
- Khaodhiar, L., Ling, P.R., Blackburn, G.L., Bistran, B.R., 2004. Serum levels of interleukin-6 and C-reactive protein correlate with body mass index across the broad range of obesity. *J. Parenter. Enteral Nutr.* 28, 410–415.
- Krivoy, A., Onn, R., Vilner, Y., Hochman, E., Weizman, S., Paz, A., Hess, S., Sagy, R., Kimhi-Nesher, S., Kalter, E., Friedman, T., Friedman, Z., Bormant, G., Trommer, S., Valevski, A., Weizman, A., 2017 Dec. Vitamin D supplementation in chronic schizophrenia patients treated with clozapine: a randomized, double-blind, placebo-controlled clinical trial. *EBioMedicine* 26, 138–145.
- Kunisawa, J., Kiyono, H., 2013. Vitamin-mediated regulation of intestinal immunity. *Front. Immunol.* 4, 189.
- Reich, K.M., Fedorak, R.N., Madsen, K., Kroeker, K.L., 2016. Role of vitamin D in infliximab-induced remission in adult patients with Crohn's disease. *Inflamm. Bowel Dis.* 22, 92–99.
- Sandhu, K.V., Sherwin, E., Schellekens, H., Stanton, C., Dinan, T.G., Cryan, J.F., 2017. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. *Transl. Res.* 179, 223–244.
- Severance, E.G., Gressitt, K.L., Stallings, C.R., Origoni, A.E., Khushalani, S., Leweke, F.M., Dickerson, F.B., Yolken, R.H., 2013. Discordant patterns of bacterial translocation markers and implications for innate immune imbalances in schizophrenia. *Schizophr. Res.* 148, 130–137.
- Shivakumar, V., Kalmady, S.V., Amaresha, A.C., Jose, D., Narayanaswamy, J.C., Agarwal, S.M., Joseph, B., Venkatasubramanian, G., Ravi, V., Keshavan, M.S., Gangadhar, B.N., 2015. Serum vitamin D and hippocampal gray matter volume in schizophrenia. *Psychiatry Res.* 233, 175–179.
- Sikoglu, E.M., Navarro, A.A.L., Starr, D., Dvir, Y., Nwosu, B.U., Czerniak, S.M., et al., 2015 Jun. Vitamin D3 supplemental treatment for mania in youth with bipolar Spectrum disorders. *J. Child Adolesc. Psychopharmacol.* 25 (5), 415–424 (cited 2017 Jan 28).
- Stojanovic, A., Martorell, L., Montalvo, I., Ortega, L., Monseny, R., Vilella, E., Labad, J., 2014. Increased serum interleukin-6 levels in early stages of psychosis: associations with at-risk mental states and the severity of psychotic symptoms. *Psychoneuroendocrinology* 41, 23–32.
- Thakurathi, N., Stock, S., Oppenheim, C.E., Borba, C.P.C., Vincenzi, B., Seidman, L.J., et al., 2013 Sep. Open-label pilot study on vitamin D3 supplementation for antipsychotic-associated metabolic anomalies. *Int. Clin. Psychopharmacol.* 28 (5), 275–282 (cited 2017 Jan 26).
- Turner, J.R., 2009. Intestinal mucosal barrier function in health and disease. *Nat. Rev. Immunol.* 9, 799–809.
- Weiss, S.T., 2011. Bacterial components plus vitamin D: the ultimate solution to the asthma (autoimmune disease) epidemic? *J. Allergy Clin. Immunol.* 127, 1128–1130.
- Zhang, Y., Leung, D.Y., Richers, B.N., Liu, Y., Remigio, L.K., Riches, D.W., Goleva, E., 2012. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J. Immunol.* 188, 2127–2135.