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Sleep onset insomnia, daytime sleepiness and sleep duration in relationship to *Toxoplasma gondii* IgG seropositivity and serointensity

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Abstract

Toxoplasma gondii (*T. gondii*) infects central nervous tissue and is kept in relative dormancy by a healthy immune system. Sleep disturbances have been found to precipitate mental illness, suicidal behavior and car accidents, which have been previously linked to *T. gondii* as well. We speculated that if sleep disruption, particularly insomnia, would mediate, at least partly, the link between *T. gondii* infection and related behavioral dysregulation, then we would be able to identify significant associations between sleep disruption and *T. gondii*. The mechanisms for such an association may involve dopamine (DA) production by *T. gondii*, or collateral effects of immune activation necessary to keep *T. gondii* in check. Sleep questionnaires from 2031 Old Order Amish were analyzed in relationship to *T. gondii*-IgG antibodies measured by enzyme-linked immunosorbent assay (ELISA). *Toxoplasma gondii* seropositivity and serointensity were not associated with any of the sleep latency variables or Epworth Sleepiness Scale (ESS). A secondary analysis identified, after adjustment for age group, a statistical trend toward shorter sleep duration in seropositive men ($p = 0.07$). In conclusion, it is unlikely that sleep disruption mediates links between *T. gondii* and mental illness or behavioral dysregulation. Trending gender differences in associations between *T. gondii* and shorter sleep need further investigation.

Keywords

Epworth Sleepiness Scale; excessive daytime sleepiness; insomnia; Old Order Amish; sleep duration; *Toxoplasma gondii*

Introduction

Insomnia, which is defined as an individual's report of "difficulty falling or staying asleep" [1], is a major public health issue [2, 3] exerting a negative impact on occupational functioning as reflected by missed work days, difficulty concentrating, accidents and poor work performance [4]. Assessment of insomnia in sleep surveys often relies on subjective questionnaires examining how one has problems falling asleep or maintaining sleep. For example, the Pittsburgh Sleep Quality Index (PSQI) [5], is a commonly utilized self-report tool, designed to determine the presence and severity of insomnia.

Insomnia has been classically considered to be a hyperarousal disorder [6–8]. Among other molecules involved in sleep regulation and dysregulation, dopamine (DA) is a key neurotransmitter involved in up-regulating arousal [9–13], and thus conceptualized as contributing to insomnia and shorter sleep duration [14]. DA also plays a role in the circadian regulation of sleep, which if dysregulated, can also contribute to insomnia complaints [15–17].

Toxoplasma gondii (*T. gondii*) is a common latency-establishing neurotropic pathogen in the immunocompetent intermediate hosts (any warm-blooded animal, including humans). There is a high prevalence of *T. gondii* infection, with almost one-third of the world's population [18] and about 11% of the United States population [19] being infected. As raw meat may contain *T. gondii* tissue cysts, as well as raw vegetables, or water supply may be contaminated with *T. gondii* oocysts from cat feces; eating undercooked meat and/or drinking contaminated water is frequently associated with disease in humans [20].

Toxoplasma gondii can produce DA in the inhabited tissues, including the central nervous system (CNS). It has two tyrosine hydroxylase enzymes with unusual substrate specificity. These enzymes can each convert both phenylalanine to tyrosine and tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) [21], resulting in high levels of DA in *T. gondii* tissue cysts in the brain [22].

Many studies, including large meta-analyses, have found an association between *T. gondii* infection and psychiatric disorders such as schizophrenia [23, 24], although a recent cohort study yielded negative results [25]. Of interest, we know that autoantibodies binding the N-methyl-D-aspartate receptors may underlie alterations in the function of glutamate receptors as well as cognitive dysfunction in schizophrenia, and that neurotropic pathogen exposure can boost autoimmunity, further increasing systemic inflammation, blood-brain barrier permeability and gut permeability [26]. In addition, significant associations have also been reported between *T. gondii* and mood disorders, such as bipolar disorder [27, 28]. Moreover, significant associations have been found between history of suicide attempt and *T. gondii* immunoglobulin G (IgG) titers [29–34] or seropositivity [32–35]. A recent cohort study

identified a statistical trend of an association between *T. gondii* seropositivity and subsequent suicide attempt, while all associations between *T. gondii* seropositivity and psychiatric illnesses were negative [25]. Similarly, *T. gondii* seropositivity was recently found to be significantly related to acoustic startle latency (ASL) in posttraumatic stress disorder (PTSD) subjects, specifically demonstrating longer startle latency in PTSD subjects [36].

Sleep abnormalities have an increased prevalence and severity in patients with psychiatric conditions [37, 38], including those previously linked with *T. gondii*. For instance, patients with schizophrenia have a markedly increased prevalence of sleep problems [39–43]. Insomnia has also been related to increased suicide rate [44] and persecutory delusions [45, 46] in patients with schizophrenia. Car accidents were previously associated with both chronic *T. gondii* infection [47–49], and with sleep disorders [50]. Thus, these studies raise the question of the possibility that sleep disturbances may mediate the link between *T. gondii* infection and mental illness, suicidal behavior and increased risk of car accidents. For this to be conceivable, *T. gondii* serointensity or seropositivity should positively relate to sleep disruption. Yet, no previous study to our knowledge has investigated the *T. gondii*-sleep association.

Thus, we tested the hypothesis that insomnia, daytime sleepiness and sleep duration are associated with *T. gondii* seropositivity. We examined this potential association in a convenience sample in the Old Order Amish in Lancaster, PA, USA; a population with a high prevalence of *T. gondii* seropositivity [51].

Materials and methods

We used data from the Amish Wellness Study, a study that was initiated in 2010 as part of the cardio-metabolic screening program for the adult population of the Amish community in Lancaster County, PA, USA. The Old Order Amish individuals recruited for our study were contacted through active engagement by the personnel of the Amish Research Clinic of the University of Maryland, Baltimore, located in Lancaster, PA, USA, and a “Wellmobile” (an RV allowing recruitment to occur in a naturalistic setting). Exclusion criteria included: age <18 years and not belonging to the Old Order Amish community. The guidelines used for investigation were in accordance with the most updated versions of the Declaration of Helsinki. The protocol was approved by the University of Maryland, Baltimore Institutional Review Board.

Informed consent was obtained after a full explanation of the study. Our study sample consisted of 2031 participants, including 1182 women (58.19%) and 849 men (41.81%). Participants ranged in age from 18 to 90 years old, with a mean age of 43.96 ± 17.03 years. Age was transformed to a binary variable by dividing the sample into two groups, i.e. above and below the median age of the sample, which was 44 years.

Excessive daytime sleepiness (EDS) was measured via the Epworth Sleepiness Scale (ESS) [52, 53]. We used a score of ≥10 on the ESS as the cutoff point for determining EDS.

We selected three questions that targeted sleep onset insomnia. One question was “difficulty falling asleep within 30 min”, which previously has been used as an insomnia indicator [5, 54, 55]. The second question was “difficulty falling asleep”, which has also been previously used as an insomnia indicator in previous studies [56–58], and was analyzed both as a categorical and as a binary variable. The third question (“number of min it takes to fall asleep”) we analyzed was a continuous variable, which has been previously used as an insomnia marker in several other studies [59–61].

We used the above-mentioned three variables to determine sleep onset latency. There were 1709 participants who responded to the question about “difficulty falling asleep within 30 min”, however, only 1703 observations, (724 men and 979 women), could be used for analysis because six people did not have *T. gondii* serologic results available. Similarly, 2031 Amish adults replied to “number of minutes it takes to fall asleep” and “difficulty falling asleep”. Out of the total data set, only 318 observations, including 120 men and 198 women, could be used for analysis of “difficulty falling asleep”, and only 303 observations, including 118 men and 185 women, could be used for analysis of “number of minutes it takes to fall asleep” because *T. gondii* serologic results were available only for these subjects.

To measure sleep duration, participants were asked to report “the number of hours they sleep at night” on the same self-reported questionnaires that were used for the sleep latency variables. A sample of 309 subjects, including 118 (38.1%) men and 191 (61.8%) women, had adequate data for sleep duration analysis. The sample was limited to this number by the availability of *T. gondii* serology results in subjects answering this sleep duration question.

At the enrollment visit, we obtained medical and family histories and scheduled a visit for a fasting blood draw. The sites used for drawing fasting blood samples included Amish Research Clinic, mobile clinic (“Wellmobile”), or the houses of Amish individuals or families. Plasma was separated by centrifugation of the blood samples for 25 min at 400 *g* and at 4°C. Plasma was then stored at −80°C.

To measure IgG seropositivity and serointensity, enzyme-linked immunosorbent assays (ELISA) (IBL International, Männedorf, Zürich, Switzerland) were used at the University of South Florida College of Nursing Biobehavioral lab located in Tampa, FL, USA, which measures levels of IgG to whole *T. gondii* tachyzoites. Standards for validation were used for all assays. To define the serologic status, we used an IgG predetermined cutoff value as reported by the manufacturer of the kit. The cutoff standard value to which the optical density (OD) was compared was 10 IU/mL. The mean coefficient of variation was 7%. The assays were re-run to confirm the status of samples that yielded equivocal results, i.e. within 20% of the cutoff OD value. Graph Pad Prism software and a cubit spline method were used for quantitative analysis by plotting the ODs of the standards against their concentrations. Then from the standard curve, the concentrations of the samples were determined. IgM antibody titers were not measured in this sample. When the ELISA results indicated an equivocal concentration of *T. gondii* antibody (8 to 12 IU/mL), we repeated the ELISA. When the second ELISA remained in the equivocal range or showed a level <8 IU/mL, the data were considered negative. If the second ELISA showed a concentration in the positive

range (>12 IU/mL) the data were considered positive. The maximum dilution was 1:20 for some of the highest values required to ensure accurate results.

Because the cellular immune response may mediate, moderate or confound the associations between *T. gondii* and sleep, we measured plasma neopterin concentration [62], a marker of cell-mediated immunity and oxidative stress, produced as a consequence of immune system activation through interactions among macrophages, granulocytes and T helper 1 (Th1) lymphocytes [63]. The concentrations of plasma neopterin were determined utilizing ELISA (BRAHMS GmbH, Hennigsdorf, Brandenburg, Germany) in accordance with the instructions from the manufacturer; 2 nmol/L neopterin was the sensitivity of the test. Intra-assay coefficients of variation ranged from 1.47% to 9.07%, while inter-assay coefficients of variation ranged from 3.03% to 10.14% [64].

Toxoplasma gondii seropositivity and serointensity were analyzed for the three different sleep onset latency variables in five different models including: Model 1 – before adjustment for any covariate; Model 2 – with adjustment for age and sex; Model 3 – with adjustment for age, sex and body mass index (BMI); Model 4 – with adjustment for age, sex and log-transformed neopterin concentration and Model 5 – with adjustment for age, sex, BMI and log-transformed neopterin concentration. *Toxoplasma gondii*-IgG titers and neopterin values were highly skewed and non-uniformly distributed; therefore, log-transformed data were used for analysis.

Sleep duration was analyzed relative to seropositivity in four models including, (1) unadjusted for covariates, (2) adjusted for covariates including age as a continuous variable and log-transformed neopterin concentration, (3) adjusted for age as a continuous variable and month of questionnaire administration (to account for seasonal effects) and (4) adjusted for age as a continuous variable and log-transformed neopterin concentration. All of the models were adjusted for sex and age as binary variables.

The statistical methods used for analysis included linear regression [65], ranked logistic regression [66] and binary logistic regression [67]. For the analysis of sleep duration as a continuous variable relative to seropositivity, we used analysis of covariance (ANCOVA). The software we used for data analysis was the Statistical Analysis System (SAS 9.3 Copyright © 2002–2010 SAS Institute Inc., Cary, NC, USA).

Results

Among the 2031 Amish adults who participated, 1104 (54.35%) were seropositive for *T. gondii* (non-transformed mean ± standard deviation (SD) titer intensity of 72.30 ± 251.74 IU/mL and log-transformed mean ± SD titer intensity of 2.68 ± 1.88).

Neopterin levels mean ± SD in seropositives were 6.26 ± 3.06 nmol/L and in seronegatives were 6.06 ± 2.55 nmol/L. Geometric mean of log-neopterin values in seropositives was 1.74 and the 95% confidence intervals for the winsorized mean were 1.72–1.76. Geometric mean of log-neopterin values in seronegatives was 1.72 and the 95% confidence intervals for the winsorized mean were 1.70–1.74.

Sleep onset insomnia

Toxoplasma gondii seropositivity (Table 1) and serointensity (Table 2) were not significantly associated with any of the three sleep onset latency variables, using logistic regression, ranked logistic regression and linear regression, in crude and multivariate models.

Sleep duration

The average sleep duration per night was 7 h and 28 min (SD = 51.24 min). We observed an interaction between *T. gondii* seropositivity and sex trending to be significant [p = 0.070, $F_{(1, 303)} = 3.31$], in relation to sleep duration with adjustment to binary age group (≥ and <44 years; the median age). When we added to the model covariates, including age as a continuous variable, log-transformed neopterin (as a marker of inflammation) and month of year in which questionnaires were distributed (adjusting for seasonal effect), the interaction between seropositivity and sex in relation to sleep duration was still trending to be significant; specifically for models having: age as a continuous variable and log-transformed neopterin [p = 0.088, $F_{(1, 277)} = 2.93$]; age as a continuous variable and month of year [p = 0.066, $F_{(1, 301)} = 3.41$]; and finally, age as a continuous variable, log-transformed neopterin and month of year [p = 0.089, $F_{(1, 276)} = 2.91$]. Specifically, we found a statistical trend ($p < 0.10$) suggesting a possible shorter sleep duration in seropositive men than seronegative men, with an average sleep of 6.82 h (SD = 0.82 h) at night in seropositive men, compared to 7.00 h (SD = 0.84 h) in seronegative men.

Daytime sleepiness

The ESS was found to be significantly related to seropositivity [p = 0.004, $F_{(1, 1701)} = 8.31$] in the unadjusted model; however, it became insignificant in models adjusted for age and sex [p = 0.329, $F_{(1, 1698)} = 0.99$]; age, sex and BMI [p = 0.328, $F_{(1, 1688)} = 0.99$]; and age, sex, BMI and log-transformed neopterin [p = 0.394, $F_{(1, 1698)} = 0.96$].

Log-transformed neopterin was not found to have significant association with insomnia parameters ($p > 0.05$), ESS scores (p = 0.548) and sleep duration (p = 0.4226).

Discussion

To the best of our knowledge, this is the first study to evaluate the potential association between *T. gondii* and sleep. Because DA is an important component of wake-promoting physiological mechanisms [17], and because *T. gondii* has the capability to produce DA [21, 22], we hypothesized that latent *T. gondii* infection may be associated with insomnia and changes in sleep duration, as both *T. gondii* and insomnia are associated with suicidal behavior and mental illness. It was possible that sleep disturbance, in particular insomnia, could be mediating, at least in part, mental illness and behavioral dysregulation linked to *T. gondii* and represent a potentially modifiable mediator and treatment target in *T. gondii* positive individuals with mental illness and increased risk for suicidal behavior. However, our negative results do not support this concept. We did analyze *T. gondii* seropositivity and serointensity for three variables related to sleep onset latency, with and without adjusting for different covariates including age, sex, BMI and neopterin, but none of these analyses yielded any significant results.

We also hypothesized that *T. gondii* seropositivity or serointensity might be associated with EDS, measured via ESS, and changes in sleep duration. To the best of our knowledge, there are no previous studies examining the possible relationship between markers of *T. gondii* infection, sleep duration and daytime sleepiness. Specifically, while the low-grade immune activation necessary to hold *T. gondii* in check [68–72], and the associations of up-regulated inflammation with longer sleep duration [73–75], would lead to hypothesizing an increased EDS and increased sleep duration in *T. gondii*-positive individuals, the DA producing theory would lead to hypothesizing a decreased EDS and decreased sleep duration. Thus, probably because of these potential contrasting effects, we did not find any association between sleep-wake disturbance and *T. gondii* seropositivity or serointensity after adjustment for confounders. As insomnia or increased sleep are also symptoms as well as prodromes of depression, absence of a relationship between sleep-wake disturbance and *T. gondii* seropositivity or serointensity may be consistent with a possible resilience of the Amish to *T. gondii* infection, and thus, absent links between depression and parasitic infection. However, we recently found that *T. gondii* serointensity was positively associated only with current dysphoria/hopelessness and not with current anhedonia [76]. Wadhawan et al. [76] further hypothesized that the lack of an association of *T. gondii* serointensity with current anhedonia may have been the result of *T. gondii's* inherent ability to produce DA [22], whose deficiency has also been associated with anhedonia in previous studies [77]. It is also possible that, as the Amish are mostly an agrarian community and do more physical work, they might develop more sleep-pressure towards the end of the day, which could attenuate the arousing effects of extra DA produced by the parasite within the brain tissue.

Also, we identified that in males (after adjustment for age group), sleep duration was trending toward a lower sleep duration in seropositives. If statistically significant in a future larger study, perhaps using more precise methods (for example actigraphy, polysomnography), a replication of this finding may support dopaminergic mediation rather than an inflammation-based mediation, which would have led to an opposite association.

Having performed this study in the Old Order Amish, it limits to a certain degree, the generalizability of our results. Other limitations include not asking about the use of caffeinated drinks and lack of information on the accuracy of self-reported time to sleep onset in people who do not use clocks or watches, thus having potentially a different estimation of the flow of time in Amish as compared to non-Amish. We also did not consider naps and naptime, and we did not have objective measures to corroborate our results. We did not inquire or stratify people who had symptoms or history of mental illness.

However, the Amish setting of our study has major advantages considering the limited alcohol and substance use in the Amish. A reduced exposure to bright or blue light late into the evening and night in the Amish due to them having prohibition of network electric light, television, computers and cell phones (*Ordnung*), may limit secondary insomnia due to circadian phase delay present in the non-Amish samples. Other advantages include the relatively homogenous lifestyle and relatively high rate of *T. gondii* seropositivity [51]. It is also possible that insomnia is circumscribed to episodes of infection exacerbation, occurring seldom enough for patients not to include them while estimating their sleep onset difficulties. In that case, it would still be conceivable that insomnia may mediate behavioral

effects during an exacerbation. And yet, the evidence demonstrates that DA production does not require transformation of bradyzoites to tachyzoites (reactivation), as DA also occurs abundantly in the bradyzoite stage [21].

Our gender-dependent finding (with a trend for statistical significance in men only) is consistent with other studies identifying gender-specific associations of *T. gondii* infection. These include an increased impulsivity in younger (20–59 years old) infected men [78] compared to younger non-infected men, infected older (≥60 years old) men and women, regardless of their age or infection status. The above-mentioned gender-related differences were particularly evident in those with high phenylalanine:tyrosine ratio [79]. There are previous reports of an increased score of trait aggression in infected women compared to uninfected women, being also moderated by the phenylalanine:tyrosine ratio [78, 80], and of a significantly lower score in self-control and higher vigilance in infected men vs. non-infected men [81]. Reproductive implications of *T. gondii* seropositivity have also been reported in rodents, with *T. gondii*-infected male rats being preferentially chosen as mates over the non-infected males by non-infected female rats, potentially due to changes in testosterone levels [82]. Testosterone levels have also been reported to be higher in *T. gondii*-infected men and decreased in *T. gondii*-infected women [83], and this may explain the increased impulsivity and reduced self-control findings in men. Gender differences in hedonic ratings to cat urine odor in *T. gondii*-infected men vs. *T. gondii*-infected women [84] have also been described previously.

Conclusions

In contrast to our expectations, we did not find any significant association between *T. gondii* antibodies and sleep onset difficulties, as well as daytime sleepiness measures. Therefore, the associations of *T. gondii* with certain psychiatric conditions, behavioral problems with high mortality (car accidents and suicide), cognitive deficits and personality traits of aggression and impulsivity, are unlikely to be mediated by sleep problems. Nevertheless, it is possible that the superior “sleep hygiene” of the Amish as compared to non-Amish, e.g. less exposure to bright or blue enhanced artificial light in the evening, including brightly lit iPads, computer and television screens, the markedly lower use of coffee, alcohol or other substances, may blunt the hypothesized *T. gondii*-induced sleep-onset difficulties. Thus, the study would have to be repeated in the non-Amish.

A statistical trend suggested that *T. gondii*-seropositive men might have a shorter duration of sleep. This finding, if significantly replicated with more precise methods, may provide new pathophysiological insights and treatment targets for specific groups such as younger, highly impulsive, *T. gondii*-positive males potentially at risk for *T. gondii*-related car accidents and suicide.

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Table 1

Association between insomnia-related questions and *Toxoplasma gondii* (*T. gondii*) seropositivity, with multiple steps of adjustment.

Questions for sleep onset insomnia/*T. gondii* seropositivity	**"Difficulty falling asleep": ranked logistic regression; n = 318, seropositive (%) = 150 (47.16%)**	**"Difficulty falling asleep" as binary variable: logistic regression; n = 318, seropositive (%) = 150 (47.16%)**	**"Difficulty falling asleep within 30 min": ranked logistic regression; n = 1703, seropositive (%) = 953 (55.96%)**	**"No. of minutes it takes to fall asleep each night": linear regression; n = 303, seropositive (%) = 143 (47.19%)**
Non-adjusted	OR = 1.022	OR = 1.030	OR = 0.986	$\beta = -0.10194$
	95% CI = 0.661–1.582	95% CI = 0.657–1.616	95% CI = 0.800–1.216	Adj. $R^2 = -0.0002$
	p = 0.921	p = 0.896	p = 0.898	p = 0.330
Adjusted for age and sex	OR = 0.726	OR = 0.754	OR = 1.131	$\beta = -0.17720$
	95% CI = 0.454–1.161	95% CI = 0.460–1.236	95% CI = 0.906–1.412	Adj. $R^2 = 0.0273$
	p = 0.181	p = 0.263	p = 0.275	p = 0.099
Adjusted for age, sex and BMI	OR = 0.727	OR = 0.754	OR = 1.134	$\beta = -0.17531$
	95% CI = 0.454–1.163	95% CI = 0.460–1.237	95% CI = 0.908–1.417	Adj. $R^2 = 0.0282$
	p = 0.183	p = 0.263	p = 0.267	p = 0.103
Adjusted for age, sex and neopterin	OR = 0.813	OR = 0.823	OR = 1.143	$\beta = -0.08409$
	95% CI = 0.498–1.326	95% CI = 0.491	95% CI = 0.915–1.427	Adj. $R^2 = 0.0594$
	p = 0.406	p = 0.459	p = 0.239	p = 0.395
Adjusted for age, sex, BMI and neopterin	OR = 0.821	OR = 0.829	OR = 1.146	$\beta = -0.08181$
	95% CI = 0.503–1.341	95% CI = 0.494–1.392	95% CI = 0.917–1.433	Adj. $R^2 = 0.0567$
	p = 0.431	p = 0.478	p = 0.230	p = 0.409

Table 2

Relationship between *Toxoplasma gondii* (*T. gondii*) serointensity and sleep onset latency variables.

***T. gondii* serointensity**	**"Difficulty falling asleep": ranked logistic regression; n = 318, seropositive (%) = 150 (47.16%)**	**"Difficulty falling asleep" as binary variable: logistic regression; n = 318, seropositive (%) = 150 (47.16%)**	**"Difficulty falling asleep within 30 min": ranked logistic regression; n = 1701, seropositive (%) = 953 (55.96%)**	**"No. of minutes it takes to fall asleep each night": linear regression; n = 303, seropositive (%) = 143 (47.19%)**
Non-adjusted	OR = 1.032	OR = 1.022	OR = 0.982	β = −0.01630
	95% CI = 0.919–1.160	95% CI = 0.907–1.153	95% CI = 0.929–1.038	Adj. R^2 = −0.0022
	p = 0.595	p = 0.719	p = 0.516	p = 0.560
Adjusted for age and sex	OR = 0.924	OR = 0.919	OR = 1.017	β = −0.04149
	95% CI = 0.811–1.053	95% CI = 0.802–1.054	95% CI = 0.958–1.078	Adj. R^2 = 0.0250
	p = 0.234	p = 0.226	p = 0.583	p = 0.155
Adjusted for age, sex and BMI	OR = 0.924	OR = 0.920	OR = 1.017	β = −0.04023
	95% CI = 0.812–1.053	95% CI = 0.803–1.054	95% CI = 0.959–1.079	Adj. R^2 = 0.0257
	p = 0.237	p = 0.229	p = 0.577	p = 0.168
Adjusted for age, sex and neopterin	OR = 0.973	OR = 0.954	OR = 1.019	β = −0.01142
	95% CI = 0.848–1.116	95% CI = 0.826–1.102	95% CI = 0.960–1.081	Adj. R^2 = 0.0576
	p = 0.695	p = 0.522	p = 0.536	p = 0.674
Adjusted for age, sex, BMI and neopterin	OR = 0.976	OR = 0.956	OR = 1.019	β = −0.01054
	95% CI = 0.851–1.119	95% CI = 0.828–1.105	95% CI = 0.961–1.081	Adj. R^2 = 0.0549
	p = 0.725	p = 0.543	p = 0.530	p = 0.699