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Association of complement component C4 with conversion to psychosis among adolescents at clinical high risk of psychosis

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2017

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Abstract

Association of complement component C4 with conversion to psychosis among adolescents at clinical high risk of psychosis

By Rutika Raina

Introduction- Schizophrenia is a severe psychiatric disorder, heritable and mechanistically complex in nature. Early genetic associations suggested variation in the Major Histocompatibility Complex (MHC) locus. Recent studies have found evidence to suggest an association between different levels of risk for development of schizophrenia due to copy number variation in C4A and C4B expression (1). The increasing interest for the detection and treatment of schizophrenia has led to focused research on the pre-onset period (prodromal) as the window of opportunity for preventive intervention.

Methods- Seventy-eight individuals from the North American Prodromal Longitudinal Study who met clinically high risk (CHR) criteria were included in the study. Complement component C4 was measured in the saliva at baseline. Structure Interview of Prodromal Symptoms were measured at baseline and follow up data was collected at six, twelve, eighteen and twenty-four months. Association between complement component C4 at baseline and conversion to psychosis at the end of two years was assessed using logistic regression model controlling for age, sex, race and income status.

Results- Cases and controls did not vary on demographics at baseline. Baseline levels of complement component C4 did not predict conversion to psychosis of individuals at CHR for psychosis (OR= 0.96, 95% CI= (0.85, 1.08), p-value= 0.50). The most common positive symptoms were unusual thought content and perceptual abnormalities while the most common negative symptom was occupational functioning. Disorganization symptoms were witnessed less frequently except for trouble with focus and attention while all other general symptoms were witnessed more than 50% except for motor disturbances.

Conclusions- Theses findings suggest that there is no significant association between C4 as a biomarker in the underlying mechanism involved with the risk of converting to schizophrenia among youth at CHR of psychosis. Future studies with larger samples are needed to confirm and extend these results.

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1. INTRODUCTION

Schizophrenia is considered to be amongst the most devastating diseases with a lifetime prevalence of approximately 1% (2). Treatment of psychotic disorders usually involves antipsychotic medications but often lacks in psychosocial interventions such as family and cognitive behavioural therapy and case management. It is very rare to find sustained high-quality continuing care, unlike with cancer, heart disease and diabetes (3).

One of the most characteristic features of schizophrenia and related disorders is psychosis. It is defined as the presence of hallucinations (without insight into their pathologic nature), delusions, or both hallucinations without insight and delusion as defined by the American Psychiatric Association as well as the World Health Organization (4). Psychosis has been associated as a disruptive symptom of many neurodevelopmental, psychiatric, neurologic and medical conditions which has made it an important target of evaluation and treatment in neurologic and psychiatric practice (4).

The complement system is a component of the plasma and is an assembly of proteins found in the blood and body fluids and on cell surfaces (5). It is actively involved in mediating humoral immunity. Recent studies have shown evidence of active participation of the complement system in mediating acute inflammatory response in the body and defending the host against pathogens (5). Amongst the many complement proteins, complement component C4 is responsible for the activation of the initial step in the classical pathway of complement activation and humoral defence. It is determined by C4A and C4B, its two allotypes (6). The human C4 gene has a very complex structure, with varying copy numbers for each of its structural features. There has been compelling evidence of C4 being a genetic marker of SCZ. The structural variants of C4 could lead to altered neural C4A expression which lead to loss of important synaptic connections in the brain. While one line of evidence raises the question of

protective effects of C4B/C4S on the schizophrenia-C4 relationship, the other line of evidence speculates the deleterious effects of C4A/C4L on this relationship. Complement C4 has gathered attention given its role in neuroimmune interactions, synaptic pruning, and association with neuropsychiatric illness, especially schizophrenia and to a lesser degree, autism (7, 8). Overall, recent evidence of increased neural mRNA expression of C4 in patients as compared to controls has demonstrated that C4 locus could be a major driver of the association between SCZ and the xMHC (Extended Major Histocompatibility locus). Many early serological studies, genetic association studies and post-mortem brain expression analysis have bolstered the involvement of complement C4 in the pathogenesis of schizophrenia (9). The complement pathway also mediates innate and acquired immunity, which could suggest additional mechanisms of pathogenesis. Thus, it is necessary to advocate additional studies of complement function in order to assess the role of components of the complement system in schizophrenia (9).

The Schizophrenia Prodrome

Early detection and prevention of schizophrenia around the time of psychosis onset would provide a window of opportunity for preventive intervention (10). By evaluating on our proposed question, we could identify predictors and mechanisms of conversion to psychosis among such individuals ascertained to be in a clinical high risk or prodromal clinical state. These are critical steps in the search for preventive strategies and providing early intervention services to reduce the burden of psychosis on individuals, their family and the society (10).

The relationship between inflammatory markers and schizophrenia have long been related to each other. For this reason, our project investigates whether complement component C4 could be used to construct a classifier that distinguishes persons with CHR risk symptoms who developed psychosis from persons with CHR symptoms who did not develop psychosis using

salivary samples from adolescents with attenuated premorbid symptoms of schizophrenia within two years following the initial biomarker collection.

2. LITERATURE REVIEW

2.1 The Immune system

The term “immunity” was taken from the Latin word “immunis” which meant exempt; thus referring to the state of protection from infectious disease (11). The immune system constitutes of a wide variety of cells and molecules, who work towards recognising and responding to invading pathogenic microorganisms and cancer. The defence system is divided into two types of immune responses; 1) innate immunity and 2) adaptive immunity (11). Innate immunity is the first line of defence against pathogens and is crucial for maintaining homeostasis and activating as well as regulating adaptive immunity.

The innate immune system comprises of four types of barriers namely, anatomic, physiological, phagocytic and inflammatory. The main anatomic barrier in all living vertebrates is the skin and mucous membranes which provide mechanical protection and prevent pathogen infiltration among other sensory and excretory functions (12). The innate immune system is responsible for activation of the adaptive immune system, which is slower in response but with a high degree of specificity. There are mainly two types of adaptive immune system: 1) humoral immune system which is mediated by macromolecules found in extracellular fluids such as antibodies, antimicrobial peptides and complement proteins and 2) cell-mediated immune system which is mediated by phagocytes and antigen-specific cytotoxic T lymphocytes and the release of various cytokines in response to an antigen attack. The dominating cell type of the humoral immune system are the B cells whereas the dominating cell type of the cell-mediated immune system are the T lymphocytes (11).

2.1.1 Overview of the complement system

The complement system is an essential component of the innate immune system as well as the adaptive immune response. It not only plays an important role in elimination of pathogens involving T and B cells but is also responsible for maintaining immunologic memory which helps prevent pathogenic re-invasion. Apart from its role in the immune system, the complement system is also involved in tissue regeneration, tumour growth and various human pathological states as well as age-related macular degeneration (13). The complement system can be activated by three different pathways: 1) the classical pathway (CP), 2) the lectin pathway (LP) and 3) the alternate pathway (AP). Different pattern recognizing molecules (PRMs) are responsible for activation of these pathways (14). These PRMs initiate a protease cascade leading to the formation of a C3 convertase enzyme complex and then C5a and C5b, which leads to the formation of sublytic or lytic complexes on target membrane (15, 16).

The initiation of the CP begins with binding of complement C1q to immune complexes which are composed of immunoglobulin antibodies bound to antigen. This leads to the cleavage and activation products of C4 and C2, which combine to form the C3 convertase, C4b2a, and leads to the amplification and cleavage of C3 into two fragments: C3b, which is the large fragment that covalently attached to the surface of microbial pathogens and acts as an opsonin and C3a, which is the small fragment that activates mast cells, leading to the release of vasoactive mediators such as histamine (9). The LP, on the other hand, is activated by the binding of mannose-binding lectin (MBL) to mannose residues on the pathogen surface. This in turn leads to the activation of MASP-1 and MASP-2, which leads to the activation and further cleavage of C4 and C2 to form the C3 convertase similar to the one formed in the CP. The C3 convertase forms the C5 convertase later, by joining with C3b (C4b2a3b) (17). The third pathway of complement activation is the AP which does not require antibodies or specific structures on the microbes for initiation. AP is activated by the spontaneous hydrolysis of C3, leading to the

formation of C3(H_2O). The plasma protein factor B then binds to this complex, leading to interaction of factors D, H and I through the pathway with C3b, which generates a distinct C3 convertase C3bBb which has the ability of activating more C3 (18).

Due to the critical role that the complement system plays in both innate and adaptive immune responses, its activation is tightly regulated by different mechanisms. Complement regulating proteins are integral in protecting healthy cells from destruction. Complement activation can be regulated at the level of initiation, amplification and generation of effectors such as opsonins, membrane attack complex (MAC), and proinflammatory anaphylatoxins (19). Deficiencies or excessive complement activation/ dysregulated modulation can both lead to serious consequences resulting in diseases such as Lupus, Haemolytic Uremic Syndrome (HUS), multiple sclerosis, Alzheimer's, asthma, sepsis etc. (13).

2.2 Complement component C4

The complement system is made up of over 30 components and regulators which are widely distributed in circulations and in tissues (20), including serum proteins and cell membrane receptors. Complement component C4 is one such component of the complement system which acts as a central protein in the CP and LP (21). C4 is a subunit of the C3 and C5 convertases in its activated form and the C4b fragment becomes covalently attached to the surface of pathogens and altered self-tissue during activation, acting as an opsonin marking the surface for removal (21).

The component C4 is the most polymorphic protein of the complement system. C4 has two isotypes, namely C4A and C4B, which vary in structure, copy number and their protein products tend to bind different molecular targets (1). These C4 genes are located in the major histocompatibility complex (MHC) class III region on chromosome 6 (22). Each human C4 gene consists of 41 exons and both C4A and C4B segregate in long and short genomic forms- C4AL, C4AS, C4BL, C4BS, which are further distinguished by the presence or absence of a

human endogenous retroviral (HERV) insertion that lengthens C4 from 14-21 kb with no change in the protein sequence (1).

With C4 playing such an important role in the humoral immune response, its deficiency or excessive amount could have an adverse effect on the immune system of an individual. An excess of complement C4 could lead to the overactivation of the complement pathways, aggravating the immune response at the local tissues. On the other hand, C4 deficiency may lead to B-cell memory impairment, continuance of bacterial/ viral infections or defective processing of immune complexes (22).

2.2.1 Complement C4 and schizophrenia risk

The etiology of schizophrenia, a severe psychiatric disorder still remains unclear because of its complexity (23). However, not only is it heritable in nature but some of its pathological features include excessive loss of grey matter and reduced number of synaptic structures on neurons (1). The multi-factorial polygenic threshold model (MFPT) of schizophrenia, supported by recent genome wide association studies (GWAS) indicates to the fact that a large number of genetic risk factors can pose as discrete risk factors or can interact with environmental factors to increase the risk of the disease (9). Many studies have focused on the major histocompatibility complex region and its association with schizophrenia for a long time. Recent GWAS analyses has, however, shifted the focus on the complement pathway and more research is being done to observe if the complement gene variation could be responsible in schizophrenia pathogenesis (9).

Genetic alteration is a common change that occurs in the human genome. One of the interesting types of genetic alteration is the copy number variation (CNV), in which some genomic regions are more susceptible to the development of CNVs as compared to others,

which leads to the increase in the chance of error during the process of meiosis (24). The association of schizophrenia with complement copy number variation has been one of the most researched associations in recent times. One of the studies in 2016 was able to characterize the complex variation in the gene coding for complement component 4. Three kinds of variations were ascertained according to which, 1) the total number of copies could vary between 0 and 5, 2) each copy number could be long or short (L or S), depending on whether they contained the HERV insertion and last, 3) each copy could be of two paralogous genes, which were denoted by C4A and C4B, whose products could bind different molecular targets (25).

C4A and C4B, the two human C4 genes, manifest individual relationships with the risk of schizophrenia (1). The serum protein concentrations for C4A and C4B have been suggested to be positively correlated to the gene copy number. Further, four structural forms of C4A/C4B are commonly observed (BS, AL-BS, AL-BL and AL-AL), which had differing levels of expression and were associated with schizophrenia risk (25). To add on to this complexity, both the genes can be present in long or short forms, depending on the insertion of a human endogenous retroviral (HERV) element. This sequence insertion has been seen to be associated with increased gene expression and an increased risk is associated with the variation that increases expression of C4A (1, 9). Different lines of evidence have supported the association between complement C4A and pathogenesis of schizophrenia. These include early serological studies, post-mortem brain expression analysis as well as the genetic association studies (9). Some studies have also demonstrated the presence of C4A on neurons and synapses and have hypothesized that increased expression could lead to increased synaptic pruning, which would produce less number of synapses, most commonly observed in schizophrenia cases (25).

2.3 Schizophrenia and other psychotic disorders

The research on the clinical syndrome of schizophrenia and other psychotic disorders picked up pace in the early 1900s (26). Schizophrenia is considered to be a major psychiatric disorder associated with impairment and striking deficits in cognition and occupational functioning (27). Various movements around the world have brought attention to the unmet needs of patients with mental disorders. The global mental health (GMH) movement is one such movement which has played a significant role in raising concern about the high level of disability and stigma associated with schizophrenia, especially in low and middle-income countries (28). Schizophrenia is characterized by psychotic symptoms such as delusions, hallucinations, and though disorder as well as reduced motivation, blunted affect, poor planning and cognitive impairment (29). Even though the life prevalence of schizophrenia and other psychotic disorders is very low, these conditions are one of the major contributors to the global burden of disease (30).

According to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5), several psychopathological domains and the relative severity of these symptom domains help in characterising schizophrenia and other psychotic disorders. These relevant symptom domains include positive symptoms such as hallucinations and delusions, negative symptoms, disorganisation, cognitive impairment, motor impairment such as catatonia and mood symptoms such as depression and mania (31). DSM-5 provides the means for clinicians to rate these symptoms on a scale of 0-4 in order to use the information to provide personalised measurement-bases, collaborative treatment (31). Despite existing definitions and DSM criterion, it is not easy to make distinctions and clear boundaries between different psychotic disorders due to common symptoms shared by many of these illnesses; thus, requiring us to improve diagnostic tools and interventions.

2.3.1 Schizophrenia as a Global Health problem

People with schizophrenia are usually at an increased risk for physical comorbidities or an early death. Growing body of evidence has suggested premature mortality to be a result of inequality in health care for people with schizophrenia and other psychotic disorders (32). Various racial and socio-economic prejudices are responsible for the lack of access to proper diagnosis and treatment of mental disorders around the world. Racial disparity arises during the diagnosis and treatment of schizophrenia. For example, schizophrenia is more common among some racial groups such as black immigrants from the Caribbean to northern Europe. The reasons for this are not entirely determined, and misdiagnosis has been attributed to the cultural insensitivity on the part of the clinician (33).

The relationship between socio-economic disparity and psychotic disorders is much more complex due to a vicious circle that is established between the two. While it is known that lower income status and financial constraints act as additional stressors that increase the vulnerability to mental disorders, the more harmful consequence of a mental illness is the stigma that comes along with it which often lead to lower income, unemployment and a demotion in social status. This is why mental illnesses remain the most harmful for the poorest and the homeless, who do not have appropriate health insurance and thus have an inadequate access to healthcare services and adequate treatment (34). According to the global burden of disease study 2016, approximately 21 million people live with schizophrenia globally, which is expected to rise over the years due to population ageing. Low- and middle- income countries have majority of these people along with the highest treatment gaps (35); thus, making the disease burden a global health issue requiring urgent attention and stringent measures to eradicate the disparities appropriately.

2.3.2 Early detection and intervention in schizophrenia psychosis

The period of functional decline before full-blown psychotic symptoms first appear is usually referred to as the prodromal phase of a psychotic disorder. In order to redirect the negative trajectories of these illness', there has been an increased interest in assessing the potential for early detection and intervention during the prodromal phase (36). Another reason for focusing on the prodromal phase for effective interventions is because the nature of existing treatments tends to be palliative for these illnesses (37).

Most of the times schizophrenia is associated with a prodromal period; however, the development of the psychotic illness in patients who experience prodromal symptoms is unclear (37). This is why it is even more important to identify patients most at risk for developing a psychotic disorder. Growing evidence has been able to describe the course of the prodrome. Initially, negative or nonspecific clinical symptoms, such as depression, anxiety symptoms, social isolation and school/ occupational failure are experiences by individuals, after which basic symptoms, attenuated positive symptoms (APS) or brief, intermittent APS of moderate intensity show up. As the individuals get closer to psychosis, they are characterized by more serious APS and they exhibit pre-delusional unusual thoughts, pre-hallucinatory perceptual abnormalities or pre-thought disordered speech disturbances (26).

It is imperative to identify the critical period of intervention for faster clarification of doubts and better outcomes in predicting vulnerability of individuals who might develop psychosis (38). There is immense potential in pre-psychotic intervention as a form of indicated prevention for psychotic illness' which can help in the development of well-defined protocols to identify those at risk and treatment/ therapy options to decrease the public health burden because of these disorders and provide care and support to individuals at ultra-high and high risk of psychosis (37).

2.4 The North American Prodrome Longitudinal Study (NAPLS)

The NAPLS project is a multi-site consortium of eight programs focusing on the psychosis prodrome. The sites are located at Emory University, Harvard University, University of Calgary in Canada, University of California at Los Angeles, University of California at San Diego, University of North Carolina at Chapel Hill, Yale University and Zucker Hillside Hospital. This study was able to longitudinally follow the largest sample of prodromal subjects worldwide (N= 291) on predictors of psychosis (10). The preliminary results from this project led to a five-year prospective study "Predictors and Mechanisms of Conversion to Psychosis" funded by NIMH in 2008. This study is also referred to as NAPLS 2 and included all eight sites. The number of subjects recruited for this study were 1,044; 764 CHR participants and 280 healthy controls, making it the largest study of individuals at CHR of psychosis to date (39).

The aim of NAPLS 2 was to describe the extent of prodromal symptoms, examine the change over time of these symptoms and determine the role of these early symptoms I terms of later clinical follow-up. The results of the study suggested that the most common prodromal symptoms at baseline were positive, followed by negative and then disorganization and general. Significant improvements in all 19 SIPS symptoms was observed at each follow- up time point compared to baseline. The study recommended the need for more in depth research on individuals who not just convert but continue to show attenuated positive symptoms. More attention needs to be given to interventions that may help in improvement in quality of life for individuals with such symptoms or converted status (39).

3. METHODS

3.1 Study design and study population

The North American Prodromal Longitudinal Study (NAPLS) is a consortium of eight programs investigating the earliest phase of psychotic illness. Detailed information concerning the study design was described previously (36). Briefly, the study is funded by the National Institute of Mental Health (NIMH) and the sites are located at Emory University, Harvard University, University of Calgary, UCLA, UCSD, University of North Carolina Chapel Hill, Yale University and Zucker Hillside Hospital. Each site makes use of the Structured Interview for Prodromal Syndromes (SIPS) for evaluating and monitoring prodromal symptoms for all at risk and comparison subjects. This collaboration led to the development of the five-year prospective study "Predictors and Mechanisms of Conversion to Psychosis (NAPLS-2)" in 2008, with 764 clinical high-risk participants and 280 healthy controls.

The clinical high risk (CHR) subjects were help-seeking individuals aged between 12-35 years at recruitment, who met the criteria for one or more prodromal syndromes: (a) attenuated psychotic symptoms; (b) brief intermittent psychotic symptoms; or (c) substantial functional decline combined with a first-degree relative with a psychotic disorder, or schizotypal personality disorder in individuals younger than 18 years. The Criteria of Prodromal Syndromes (COPS) was used to assess prodromal syndromes, based on the SIPS, which was conducted by clinically-trained interviewers. Participants were excluded if they had met criteria for an Axis I psychotic disorder; treatment with antipsychotic medication was no considered for exclusion as long as it was not related to the psychotic symptoms of an individual. Healthy controls (HC) were recruited from the community and had no

personal history or first-degree relative with psychosis and did not meet criteria for any prodromal syndrome. Other criteria for exclusion for both groups included substance dependence in the past six months, neurological disorder, or full-scale IQ < 70. Non-psychotic psychiatric disorders were permitted in CHR and HC groups (39). Clinical assessments were done every 6 months and subjects were followed for up to 2 years.

3.2 Saliva Collection and C4 Assay

The present investigation includes a total of 78 subjects who were sub-sampled from the 1044 CHR participants enrolled in the NAPLS study. These subjects were assessed at baseline, and this subsample comprises of participants, as of the study midpoint, for whom at least one saliva sample for complement component C4 was available. Cases (n= 39) were defined as subjects who converted to psychosis at the end of follow up while controls (n= 39) were defined as subjects who did not convert to psychosis after the study period ended.

Saliva was stored in -20°C freezer. In preparation for the assay, samples were thawed and centrifuged at 3000g for 10 minutes to remove coagulated protein and other insoluble material. C4 was assayed in duplicate 50 µL aliquots of the clear supernatant, using materials and procedures provided by abcam's Human complement C4 Elisa kit (abcam, ab108825). Using this method, the range of salivary C4 concentrations in normal adults has been determined as 0.078 ng/mL- 20 ng/ml.

3.3 Outcome Assessment

The subjects at CHR of psychosis with prodromal symptoms who transitioned to psychosis at the end of the study period were classified into the category of converted while the subjects who did not get converted were classified into the category of not converted.

The severity of prodromal symptoms is measured by the SIPS, which is a 19-item scale with a scale of prodromal symptoms (SOPS) that ranges from 0- absent to 6- severe. The

SIPS is composed of four subscales for Positive, Negative, Disorganization and General symptoms. There are five positive symptoms which include P1- unusual thought content/ delusional ideas, P2- Suspiciousness. Persecutory ideas, P3- Grandiose ideas, P4- Perceptual Abnormalities/ hallucinations, P5- Disorganized Communication. There are six negative symptoms which include N1- Social Anhedonia, N2- Avolition, N3- Decreased expression of emotion, N4- Decreased experience of emotions and self, N5- Decreased ideational richness and N6- Occupational functioning. There are four disorganization symptoms which include D1- Odd behaviour or appearance, D2- Bizarre thinking, D3- Trouble with focus and attention and D4- Impairment in personal hygiene. There are four general symptoms which include G1- Sleep disturbance, G2- Dysphoric mood, G3- Motor disturbances and G4- Impaired tolerance to normal stress. The SIPS was administered at initial assessment and during follow-ups at 6, 12, 18 and 24 months respectively.

3.4 Statistical Analysis

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). All p-values were two-sided and p-values <0.05 or 95% confidence intervals (95% CI) that did not contain 1.0 were considered statistically significant. The subjects' characteristics at baseline were summarized and compared across cases and controls. Difference of baseline characteristics between cases and controls were assessed by pooled two-sample t-test for continuous variables and chi-square test for categorical variables. The baseline mean C4 concentration for subject ID 4.135 was above detectable range. Hence, the mean C4 concentration was set to 20.0 ng/ml for the subject, which is the maximum detectable value by abcam's Human complement C4 Elisa kit. The association of C4 concentration as a continuous variable with conversion to psychosis at the end of the study period as a binary variable was analysed using multivariable logistic regression to calculate odds ratio (OR) and their 95% CIs. Covariates were chosen based on plausibility and previous literature

and included age (in years), race, sex, and household income. This model was also used in the stratified analysis to examine the difference in associations of the C4 concentration with conversion to psychosis with weight (in pounds) as a covariate in the model. For selected categorical variables, the strata were: race- First Nations, Asian, Black, Latin America/ Central Asia and Middle East/ European, Interracial; sex- female or male; household income- >40,000, <40,000 and missing. Due to sparse data for the different categories of race variable, the variable was condensed into the following strata for the purpose of analysis- 1) First Nations/ Asian/ Interracial, 2) Black and 3) Latin America/ Central Asia and Middle East/ European.

Follow-up data was available for each of the 19 SOPS symptoms as well as the combined domain symptoms score. Graphical analysis was conducted to observe trends in the change of SOPS symptoms over time in relation with C4 concentration and conversion status. C4 concentration was recoded as a binary variable around the mean value of 2.5 ng/ml with the strata- < 2.5 ng/ml and > 2.5 ng/ml. Combined domain scores were created by adding up the scores for individual items in each of the domains; creating four domains, namely- total positive symptoms score, total negative symptoms score, total disorganization symptoms score and total general symptoms score.

4. RESULTS

C4 concentration

Baseline C4 concentration was ascertained from 77 saliva samples at baseline. One sample did not have C4 concentration in the detectable range and was given the highest detectable value of 20 ng/ml for our diagnostic kit for human complement C4. The C4 concentration had a mean of 4.26 ng/ml and a standard deviation of 4.41 ng/ml. The C4 concentration ranged from 0.07 ng/ ml to 20 ng/ml, with a slightly right skewed distribution (Figure 1, Figure 2). Within conversion status, the mean C4 concentration among the converted group was 3.83 ng/ml with a standard deviation of 3.73 ng/ml whereas the mean C4 concentration among the non-converted group was 4.69 ng/ml with a standard deviation of 5.02 ng/ml (Figure 3). The F-statistic for equality of variances was 1.81 with p-value of 0.07 > 0.05 because of which we considered the pooled t-test estimate of variance. The t-statistic was 0.86 with a p-value of 0.39 which concluded that the mean C4 concentration was not different by conversion status if subjects.

Sample characteristics

The sample consisted of 78 individuals, with 39 individuals in the control group (17 females, 22 males) and 39 individuals in the CHR group (17 females, 22 males) are referred to in Table 1. There was no significant age and weight difference between cases and controls (mean age= 18.1 years, mean weight= 152.3 lbs). The groups did not differ in race, with the majority being Latin American/ Middle eastern/ White (converted 71.8%, non-converted 69.2%) or ethnicity, with the majority being non-Hispanic (converted 89.7%, non-converted 74.4%) or marital status, with the majority being single (94.9%) or living arrangement, with the majority living with family (79.5%) or household income status >40,000$ (converted 53.8%, non-converted 38.5%). Majority of the individuals were not working (85.7%) and

were enrolled as a student (89.6%). Most of the individuals had completed some grade schooling but not completed high school (63.6%). The groups also did not significantly differ in substance use, with the majority being non-tobacco users (80.8%), non-alcohol users (61.5%) and non-cannabis users (75.6%).

The mean and standard deviations of all 19 SOPS symptoms are presented in Appendix 4. The most common positive symptoms were unusual thought content and perceptual abnormalities. For negative symptoms, this ranged from 61% witnessing occupational functioning to 20% witnessing decreased ideational richness. Except for trouble with focus and attention, disorganization symptoms were witnessed less frequently. Except for motor disturbances, all other general symptoms were being witnessed more than 50% of the time among the subjects.

Conversion status

Baseline levels of complement component C4 did not predict conversion to psychosis of individuals at CHR for psychosis (OR= 0.96, 95% CI= (0.85, 1.08), p-value= 0.50) (Table 2). Baseline levels of log transformed C4 concentration were also assessed with conversion status, which yielded similar results. The sensitivity analysis with weight (in lbs.) in the model suggested no significant difference in the association between C4 concentration and conversion status of individuals at CHR of psychosis (OR= 0.92, 95% CI= (0.80, 1.07), p-value= 0.29) (Appendix 3).

Exploratory graphical analysis of SOPS symptoms over time

The scatterplots for assessing individual's change in symptoms over time suggested that each individual had their own trajectory for symptom change for all 19 symptoms as well as the combined symptoms scores (Figure 4 to Figure 49). Missing data on SOPS symptoms over time was common for visits 12 months, 18 months and 24 months after baseline. The average change in SOPS symptoms and combined symptom scores to assess whether

symptom change trajectories differed between subjects grouped by conversion status and C4 concentration suggested almost no variation in the symptoms on both sides of the mean C4 concentration over time for non-converted subjects based on the regression line. For converted subjects, majority of the trajectories showed an overall decreasing trend in symptoms over time, except for N2, N6 and D2 symptoms, where the overall regression line seemed to go upwards over time (Figure 50 to Figure 72). The graphical analysis also suggested that each individual had their own slope and intercept, which needs to be kept in mind for future longitudinal analysis. Thus, while it was beyond the scope of the current thesis to examine longitudinal changes in symptom trajectory as a function of C4 levels, longitudinal analysis may reveal differences in symptom trajectories based on various cut-points for C4.

5. DISCUSSION

Our findings suggest that there is no significant association between C4 concentration at baseline with the conversion status after end of study period among youth at CHR for psychosis. There is little to no variance in demographic characteristics between cases and controls and our sensitivity analysis suggests no significant difference by weight in conversion status. The frequencies of the number of clinical high-risk subjects witnessing each of the 19 symptoms on the Scale of Prodromal Symptoms were observed to be the highest for positive symptoms and negative symptoms as compared to disorganization symptoms and general symptoms.

A study aimed at understanding the underlying genetics associated with the risk of development of schizophrenia suggested that the association of schizophrenia with the MHC locus mainly comes from the diverse alleles of the C4 gene, which lead to varying expression levels of C4A and C4B; with a greater expression of C4A in the brain. Their results further suggested that this increased C4 activity might explain the reduced number of synapses in the brains of affected individuals, thus making C4 a crucial part in the development of schizophrenia (1).

Our study had several strengths. First was the prospective design of the NAPLS study from which this case-control study was adapted. Case control studies are advantageous for diseases with a long latency period and schizophrenia as well as other psychotic disorders take a long period to actually manifest in an individual. Further, our study aimed at evaluating the relationship between C4 and conversion status among youth at CHR of psychosis. It is usually difficult to keep young people in studies for long periods of time.

Our study also had several limitations. First is the small sample size (n=78), which may limit generalizability of our findings. Second, our subjects who may have varied concentrations of C4

during follow- up could not be taken into consideration due to availability of salivary samples only at baseline. Third, the subjects would have been on psychiatric medications before enrolling in the study and even during the study which could have altered their quantity of saliva. This could have in turn, limited our extraction of C4 from saliva samples. An important limitation was that we only examined symptoms in relation to the mean for C4, and other cut-points may have revealed differences between groups based on C4 levels.

Severe mental illnesses such as schizophrenia have increased the strain on hospitals and health care services due to increase in the number of people living with such disorders. Further, the most common age group to be identified with schizophrenia tends to be between 25- 50 years of age; the age which is the most productive. These leads to economic deficits and burden on the individual's family to meet the needs for the treatment along with sustenance. Since the effect of these illnesses is more harmful in low- and middle- income countries, urgent public health action is required to mitigate the gaps in treatment and provide care for people suffering from mental illnesses. Recognizing the prodromal symptoms and understanding the biological processes that lead up to the conversion to psychosis is a promising path towards early intervention.

In conclusion, our findings, taken in context with those from previous studies, suggest contrasting results. Based on our graphical analysis to observe a relationship between C4 concentration and individual items on the SOPS scale over time, there may be an inverse relationship between C4 and some of the prodromal symptoms. A longitudinal analysis needs to be conducted to evaluate this relationship and validate preliminary exploratory analysis to prove or disprove our hypothesis in the future.

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7. Tables

Table 1. Characteristics of sub-sample of youth at clinical high risk of Psychosis (N= 78) at baseline, NAPLS study, 2008-2015

Characteristics	Cases (n=39) *	Controls (n= 39)	Test statistic
Age (years)[1]	18.1 (3.5)	18.1 (3.5)	0.00**
Weight (lbs.)[1]	150.3 (30.5)	152.3 (37.4)	0.23**
Sex, Female (%)	17 (43.6)	17 (43.6)	0.0
Race (%)			2.6
First Nations	1 (2.6)	1 (2.6)	
Asian	3 (7.7)	2 (5.1)	
Black	4 (10.3)	8 (20.5)	
Latin America/ Central Asia & Middle East/ European	28 (71.8)	27 (69.2)	
Interracial	3 (7.7)	1 (2.6)	
Ethnicity, Hispanic (%)	4 (10.3)	10 (25.6)	3.1
Marital Status, single never married (%)	36 (92.3)	38 (97.4)	1.4
Current living arrangement, with family (%)	30 (76.9)	32 (82.1)	3.4
Total Household income, >40,000 (%)	21 (53.8)	15 (38.5)	1.9
Education level attained (%)			3.4
Not completed high school	25 (64.1)	24 (63.2)	
High school	13 (33.3)	10 (26.3)	
College	0.0	2 (5.3)	
Technical school	0.0	1 (2.6)	
University	1 (2.6)	1 (2.6)	
Currently working, Yes (%)	6 (15.4)	5 (13.2)	0.1
Currently enrolled as student, Yes (%)	34 (87.2)	35 (92.1)	0.5
Substance use			
Tobacco use (%)			2.1
Never used	29 (74.4)	34 (87.2)	
Ever used	10 (25.6)	5 (12.8)	
Alcohol use (%)			0.2
No use	23 (59.0)	25 (64.1)	
1-4 times/month	10 (25.6)	9 (23.1)	
1-4 times/week	6 (15.4)	5 (12.8)	
Marijuana use (%)			0.1
Never used	30 (76.9)	29 (74.4)	
Ever used	9 (23.1)	10 (25.6)	

*Cases are defined as the CHR youth who got converted to psychosis at the end of follow up.

**Pooled t-test statistics are reported for age and weight. Chi- square test statistic was reported for all categorical measures

Table 2: OR of conversion status associated with covariates in the NAPLS study, 2008-2015

Covariate	OR	95% CI	p-value
C4 concentration (ng/ml) *	0.96	0.86, 1.06	0.39
Age (in years)	1.00	0.88, 1.14	1.00
Sex	1.00	0.41, 2.45	1.00
Weight (in lbs.)	1.00	0.98, 1.01	0.82
Race			
Latin America/Central Asia & Middle East/ European	Ref.	Ref.	Ref.
First Nation/ Asian/ Interracial	1.69	0.44, 6.43	0.44
Black	0.48	0.13, 1.79	0.28
Income			
>40,000	Ref.	Ref.	Ref.
<40,000	0.50	0.16, 1.61	0.25
Missing	0.56	0.20, 1.57	0.27
Fully adjusted model**	0.96	0.85, 1.08	0.50

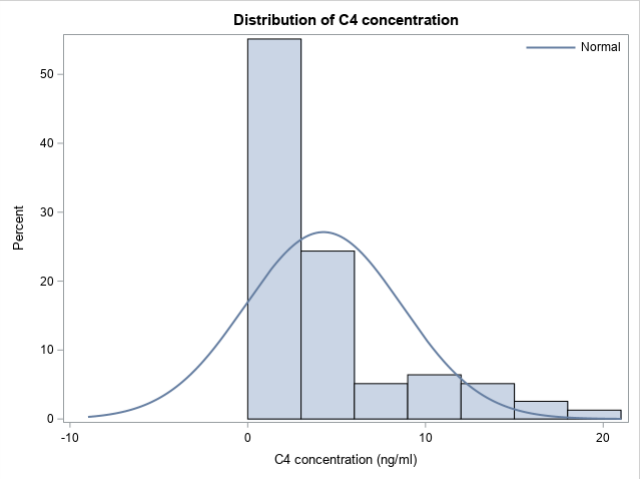
* C4 concentration is the main exposure which was collected from salivary samples at baseline

**Fully adjusted model tests the association of conversion status with C4 concentration, controlling for age (years), sex, race and income >40,0000

[1] Age and Weight are presented as mean (std. deviation). The 25th percentile, median and the 75th percentile for age and weight are 15, 18, 20 years and 129, 145, 165 lbs respectively.

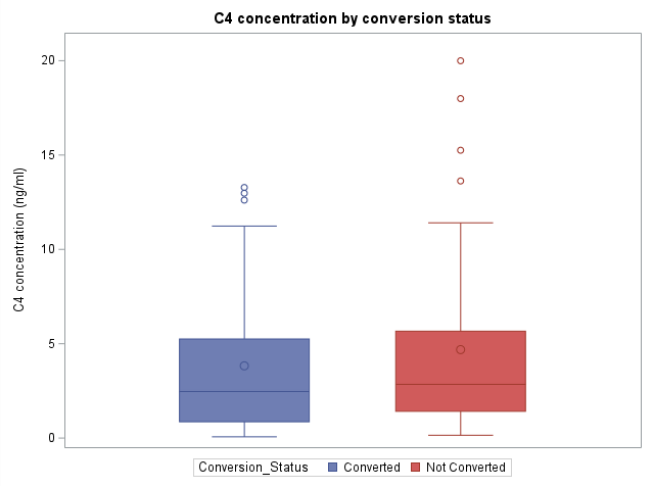
8. FIGURES

Figure 1. Distribution of C4 concentration (ng/ml)



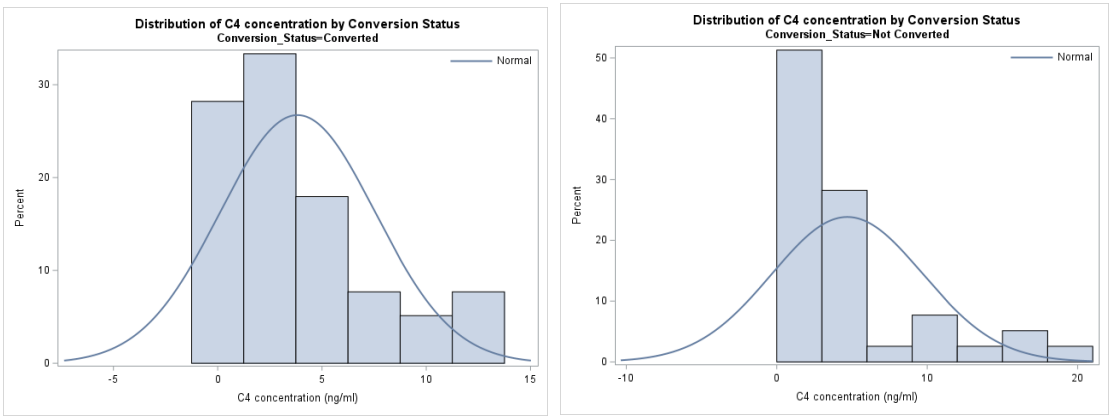
The bars represent the number of subjects with an overlaid normal distribution curve fitted to the distribution of C4 concentration.

Figure 2. Box plot for the distribution of C4 concentration by conversion status



The figure represents the median, interquartile ranges and outliers for C4 concentration by conversion status, where the blue box plot represents converted subjects and the red box plot represents non-converted subjects.

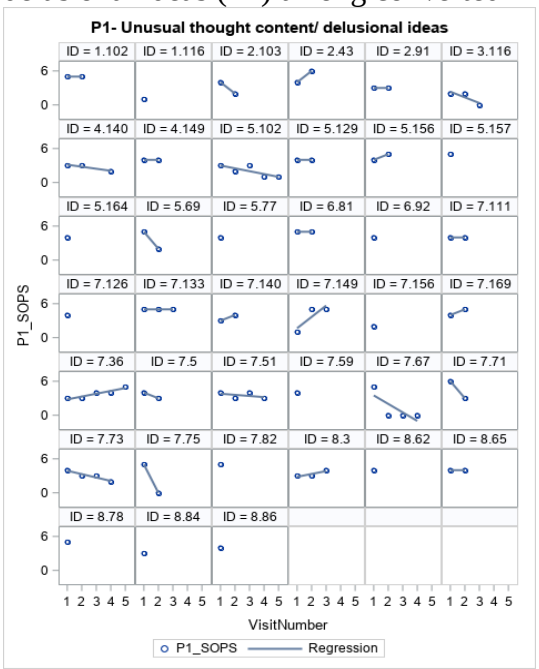
Figure 3. Distribution of C4 concentration by conversion status



The figure on the left shows the distribution of C4 concentration among converted subjects while the figure on the right shows the distribution of C4 concentration among non-converted subjects.

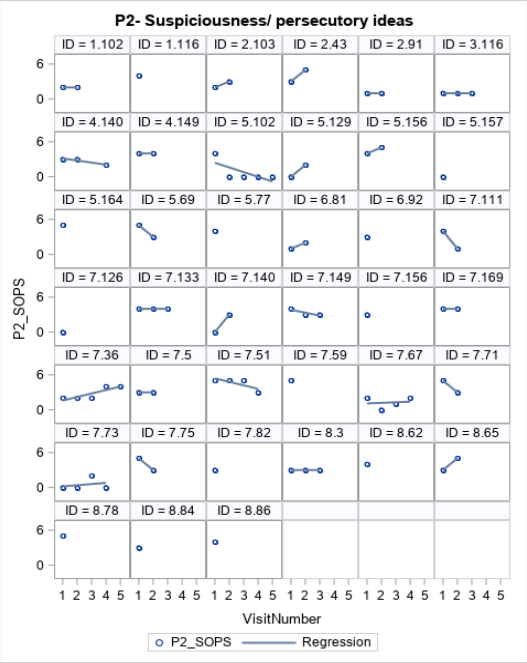
Figure 4-41. Individual changes in each of the 19 prodromal symptoms score by conversion status, over time in a sub-sample of youth from NAPLS study

Figure 4. Unusual thought content/ delusional ideas (P1) among converted



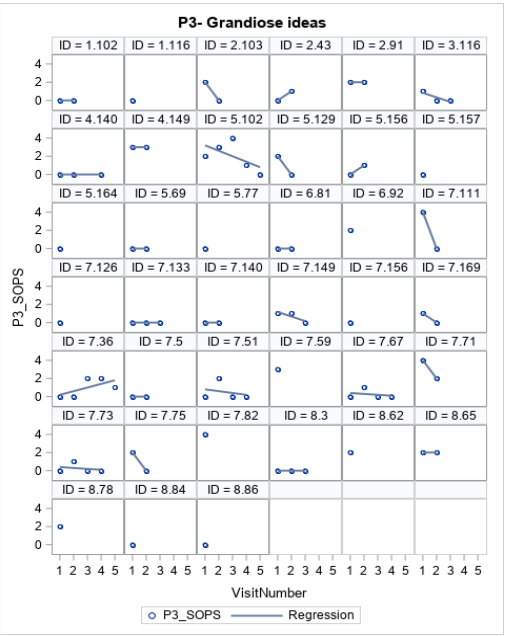
The figure represents change in P1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 5. Suspiciousness/ persecutory Ideas (P2) among converted



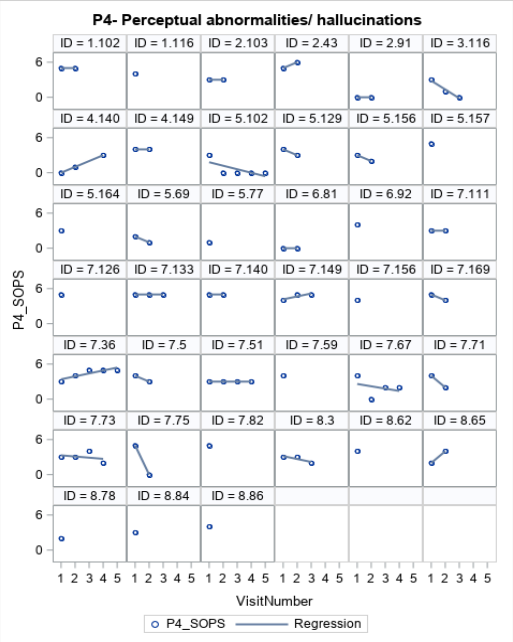
The figure represents change in P2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 6. Grandiose ideas (P3) among converted



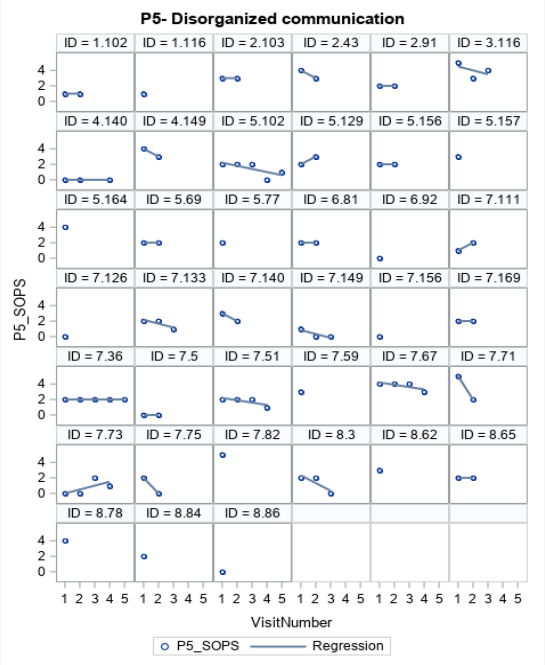
The figure represents change in P3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 7. Perceptual abnormalities/ Hallucinations (P4) among converted



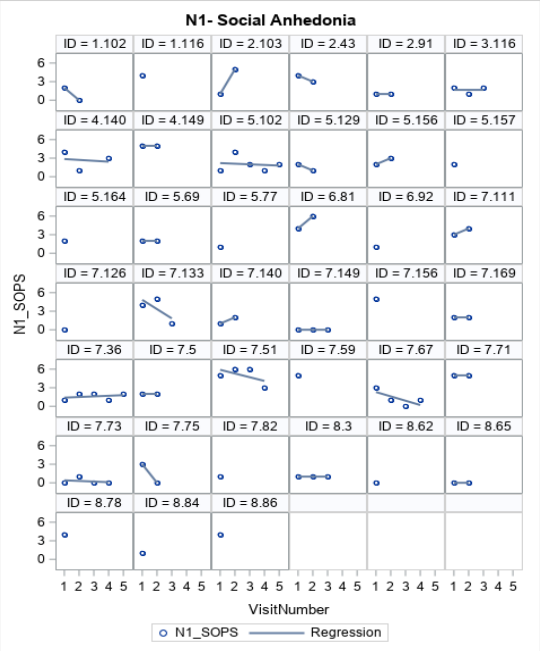
The figure represents change in P4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 8. Disorganized communication (P5) among converted



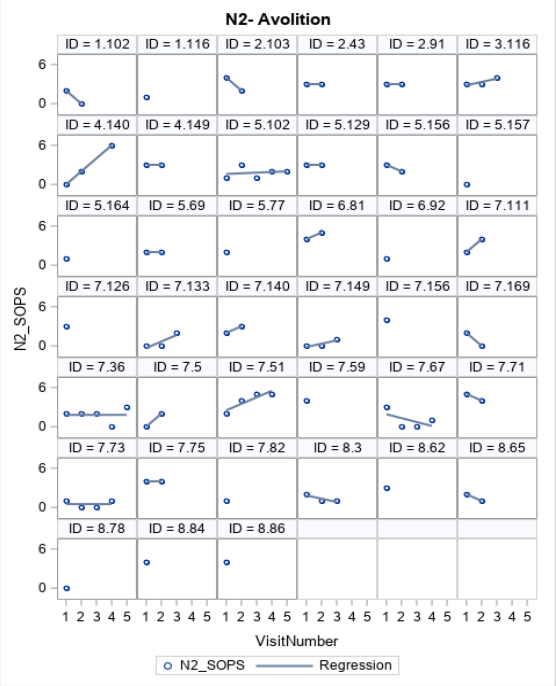
The figure represents change in P5 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 9. Social anhedonia (N1) among converted



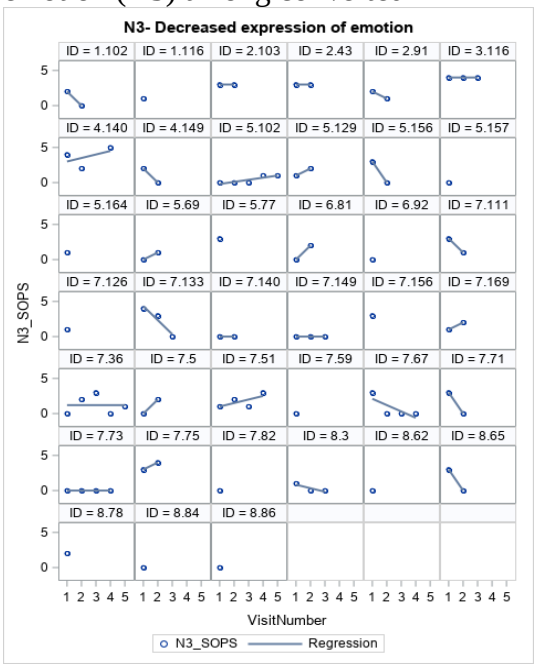
The figure represents change in N1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 10. Avolition (N2) among converted



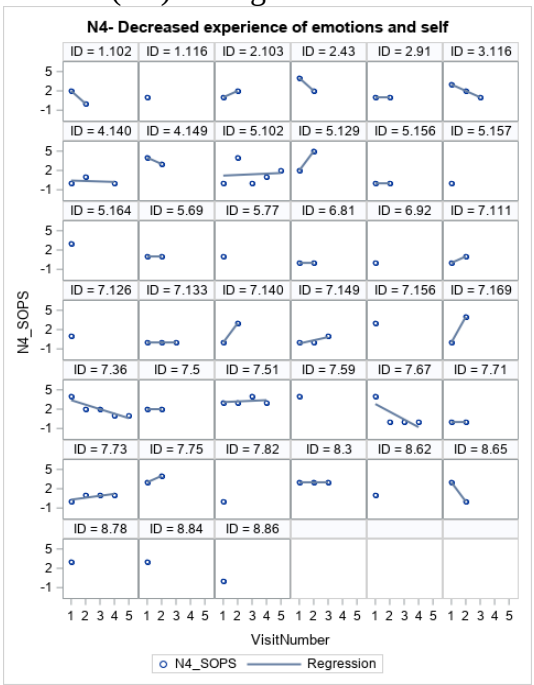
The figure represents change in N2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 11. Decreased expression of emotion (N3) among converted



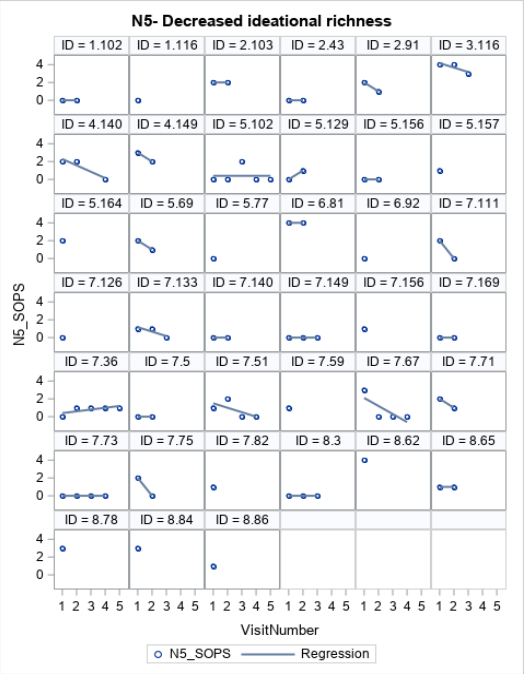
The figure represents change in N3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 12. Decreased experience of emotions and self (N4) among converted



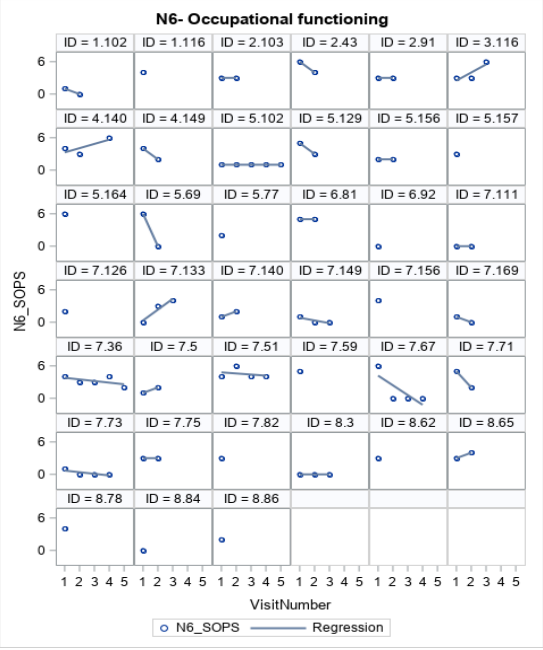
The figure represents change in N4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 13. Decreased ideational richness (N5) among converted



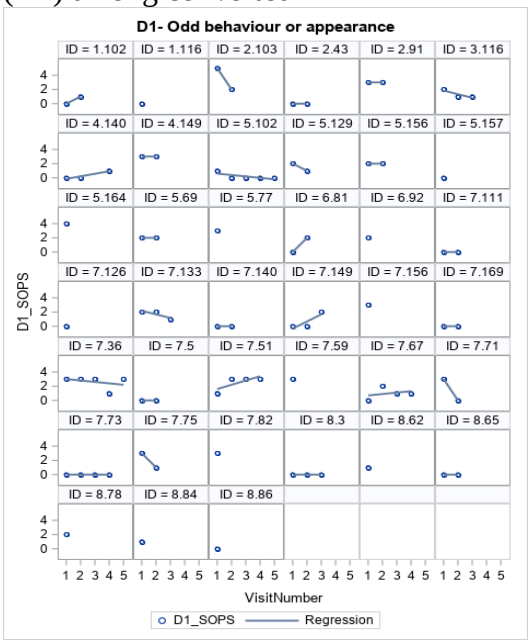
The figure represents change in N5 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 14. Occupational functioning (N6) among converted



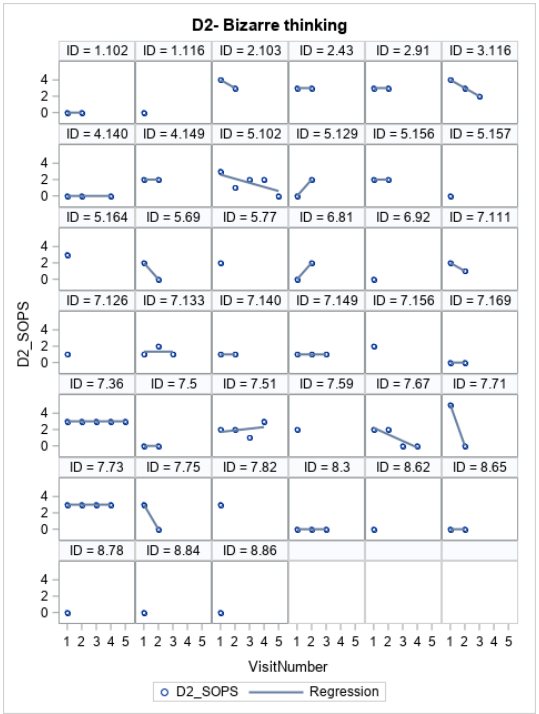
The figure represents change in N6 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 15. Odd behaviour or apperance (D1) among converted



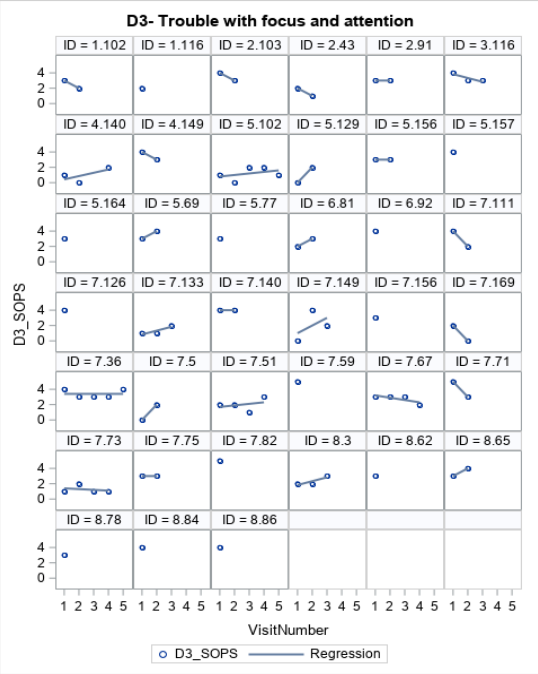
The figure represents change in D1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 16. Bizarre thinking (D2) among converted



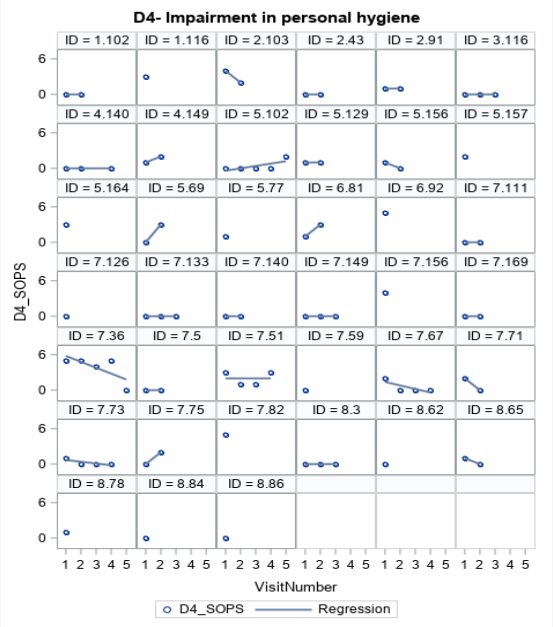
The figure represents change in D2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 17. Trouble with focus and attention (D3) among converted



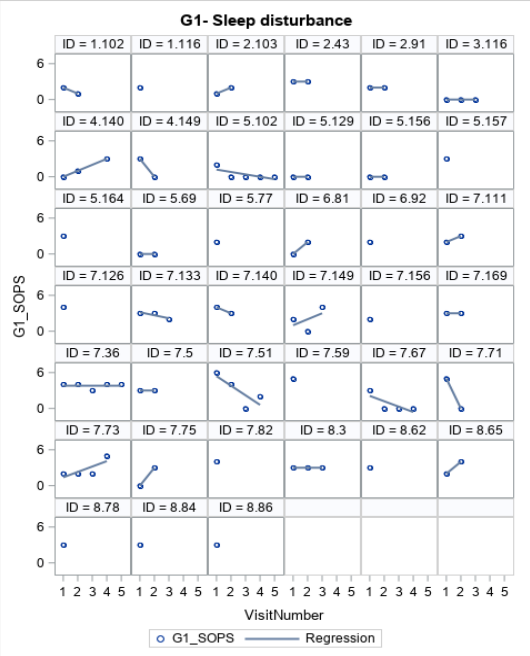
The figure represents change in D3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 18. Impairment in personal hygiene (D4) among converted



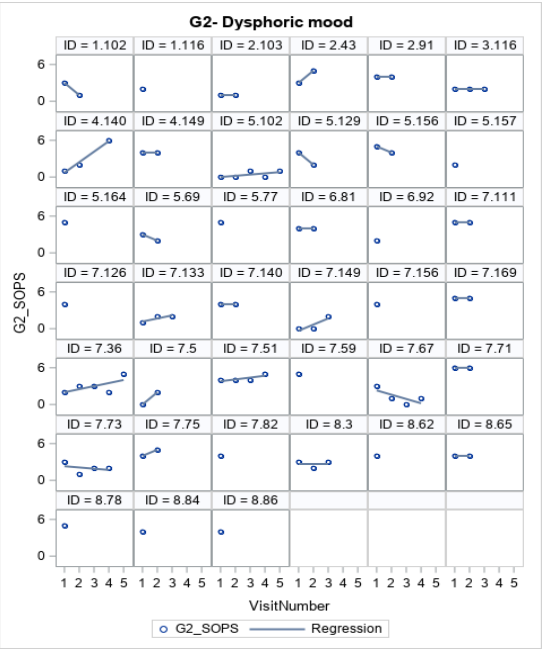
The figure represents change in D4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 19. Sleep disturbance (G1) among converted



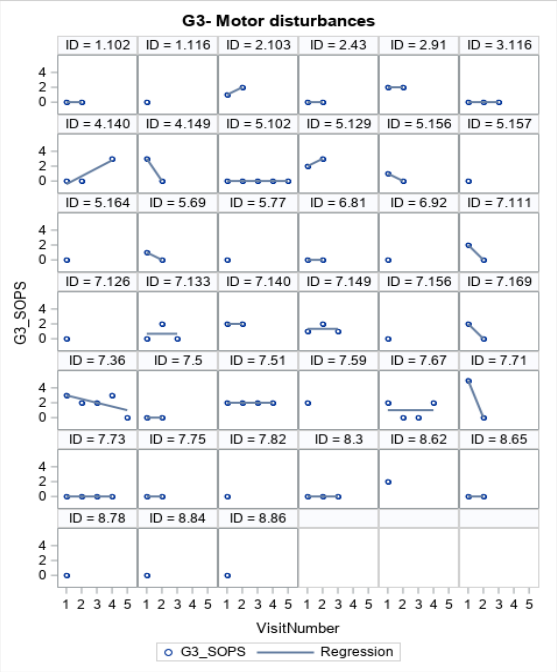
The figure represents change in G1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 20. Dysphoric mood (G2) among Converted



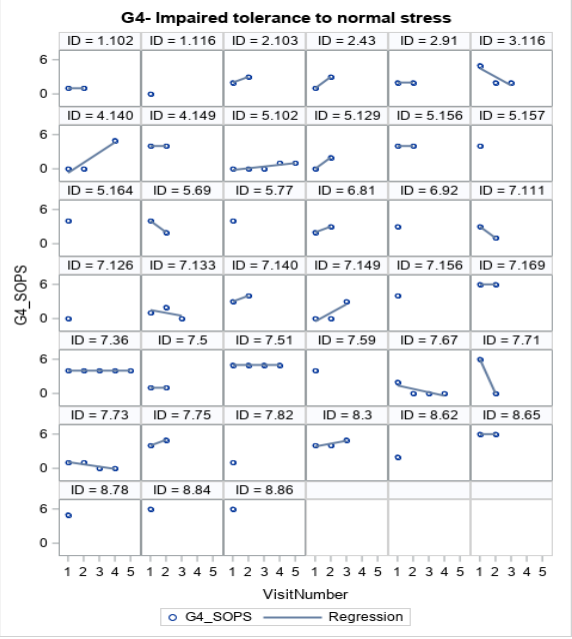
The figure represents change in G2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 21. Motor disturbances (G3) among converted



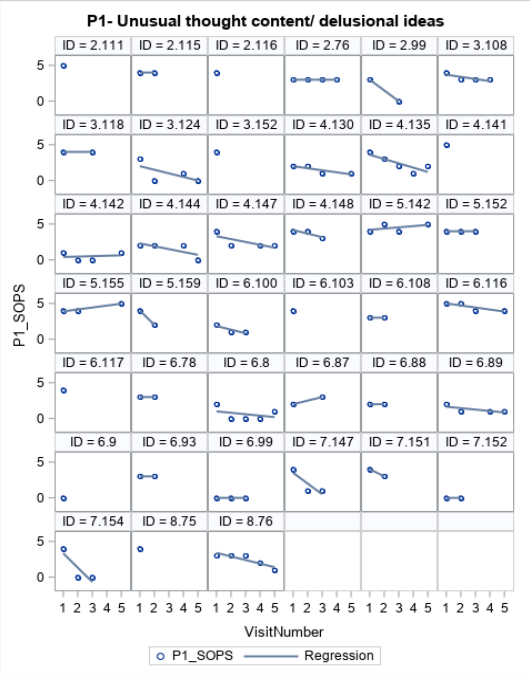
The figure represents change in G3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 22. Impaired tolerance to normal stress (G4) among converted



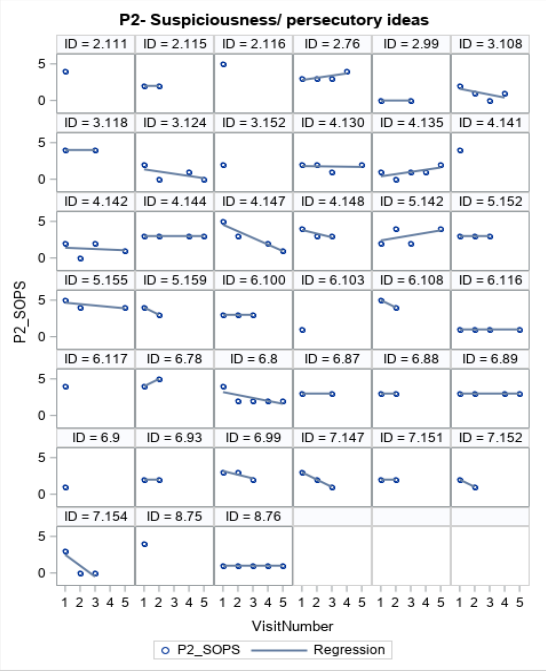
The figure represents change in G4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 23. Unusual thought content/ delusional ideas (P1) among non-converted



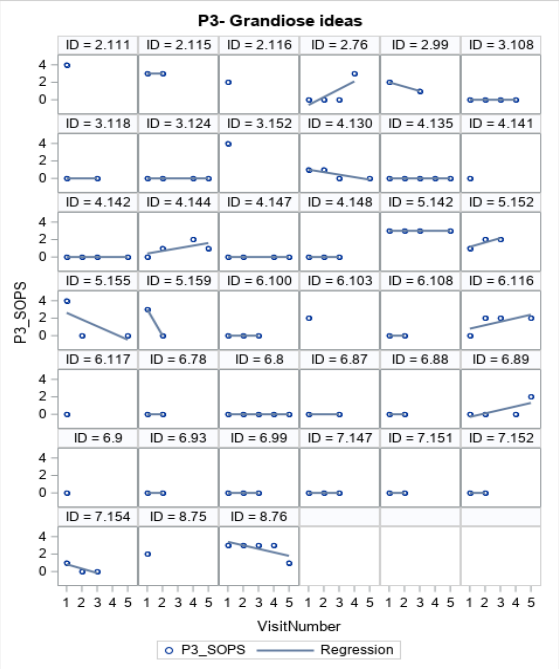
The figure represents change in P1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 24. Suspiciousness/ persecutory ideas (P2) among non-converted



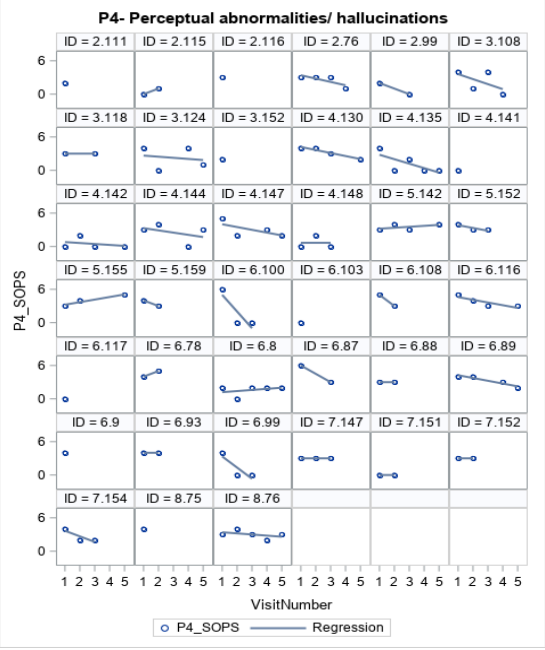
The figure represents change in P2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 25. Grandiose ideas (P3) among non-converted



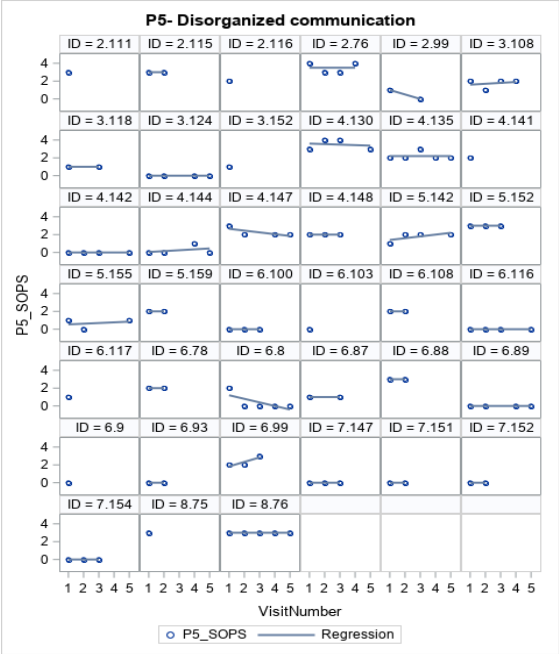
The figure represents change in P3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 26. Perceptual abnormalities/ hallucinations (P4) among non-converted



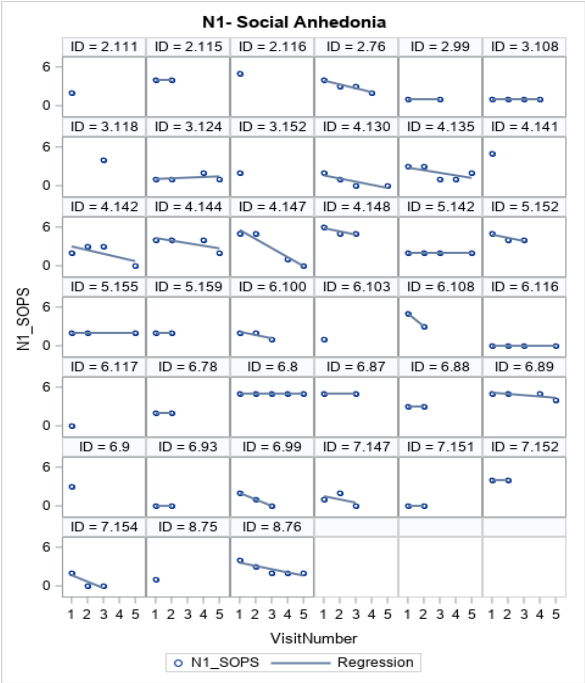
The figure represents change in P4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 27. Disorganized communication (P5) among non-converted



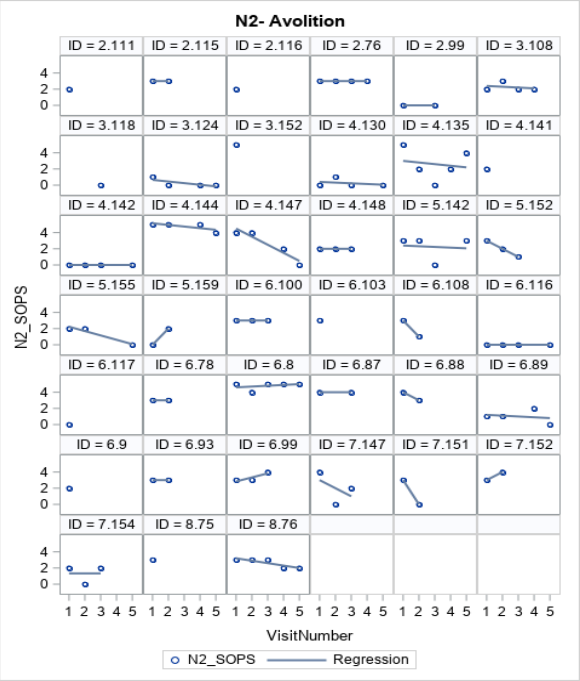
The figure represents change in P5 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 28. Social anhedonia (N1) among non-converted



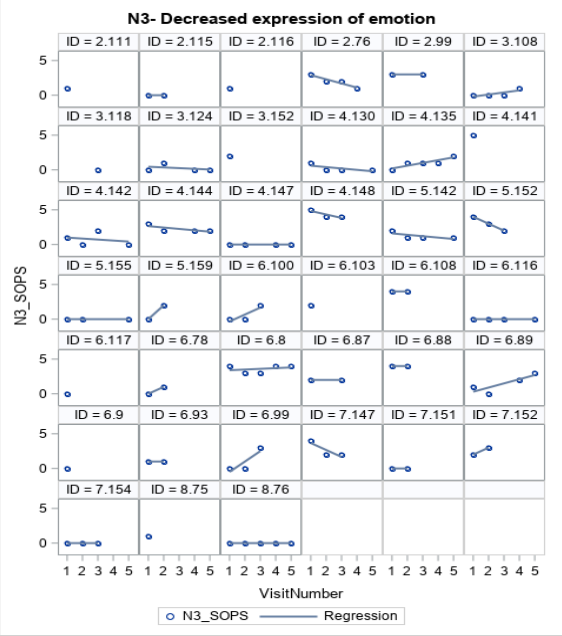
The figure represents change in N1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 29. Avolition (N2) among non-converted



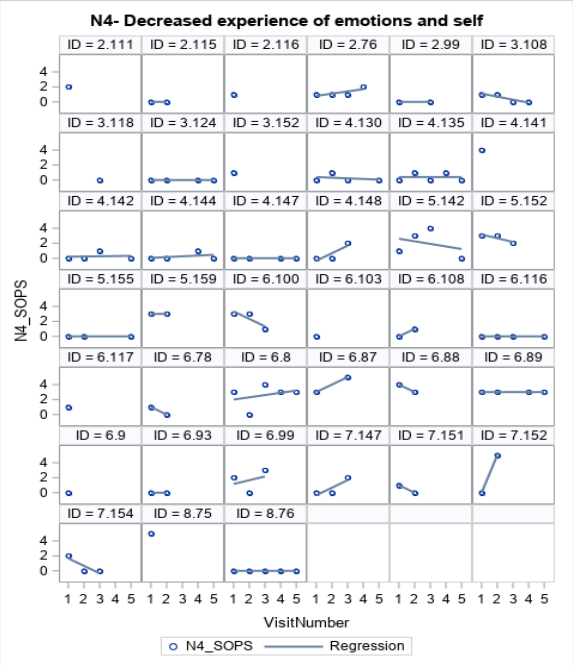
The figure represents change in N2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 30. Decreased expression of emotion (N3) among non-converted



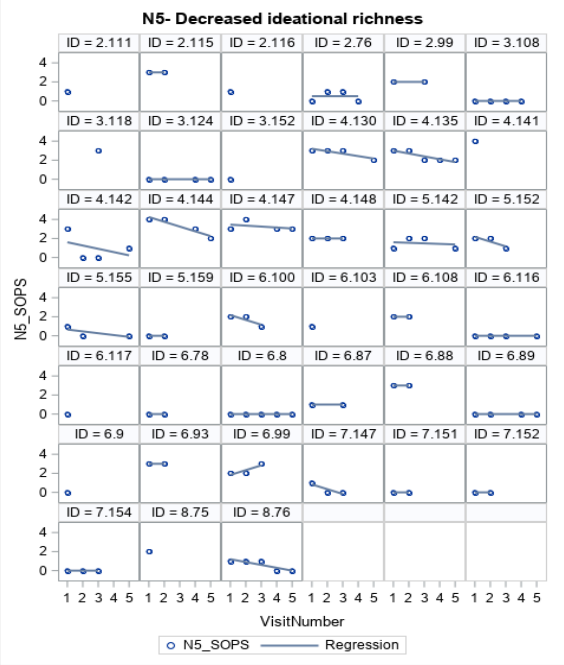
The figure represents change in N3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 31. Decreased experience of emotions And self (N4) among non-converted



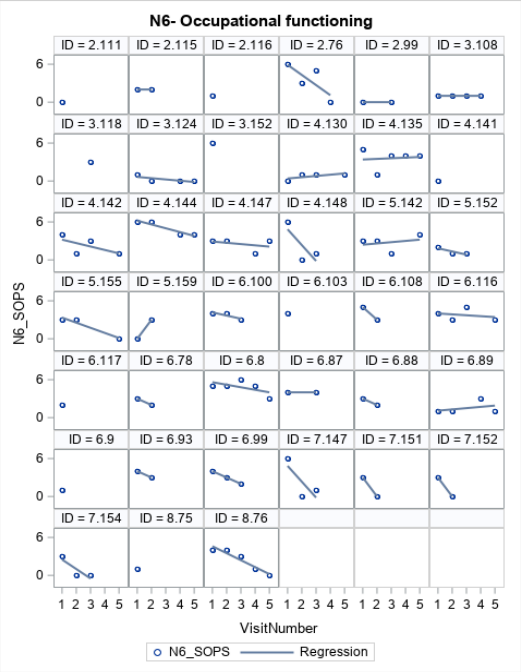
The figure represents change in N4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 32. Decreased ideational richness (N5) among non-converted



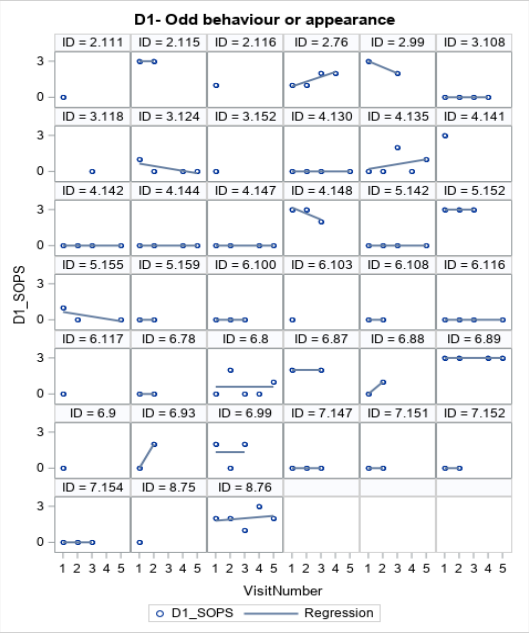
The figure represents change in N5 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 33. Occupational functioning (N6) among non-converted



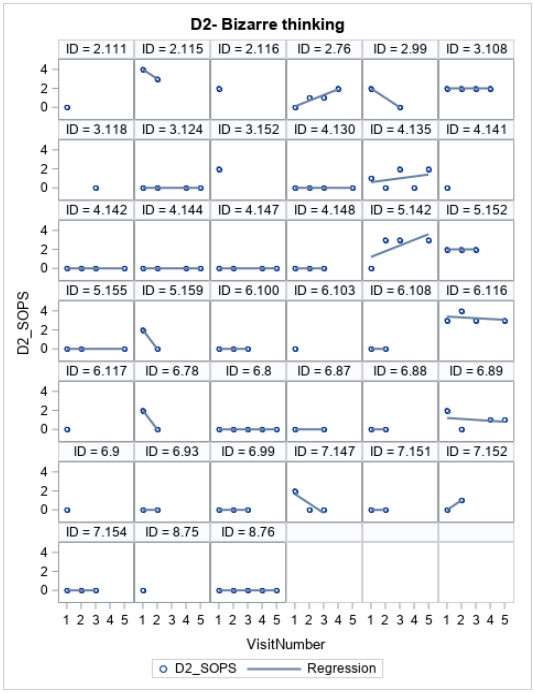
The figure represents change in N6 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 34. Odd behaviour or appearance (D1) among non-converted



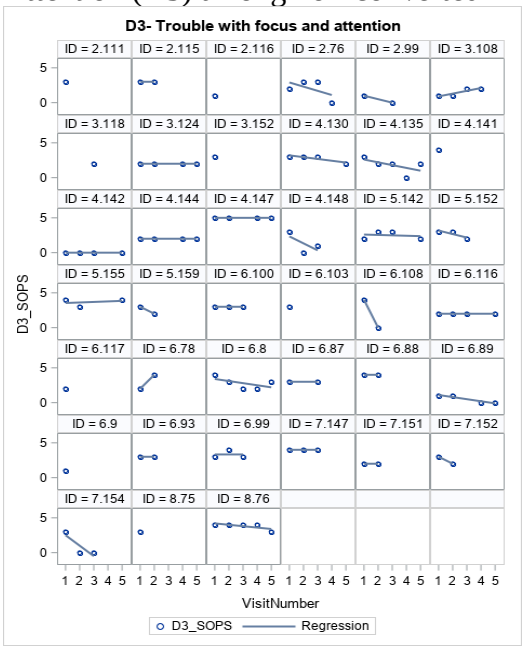
The figure represents change in D1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 35. Bizarre thinking (D2) among Non-converted



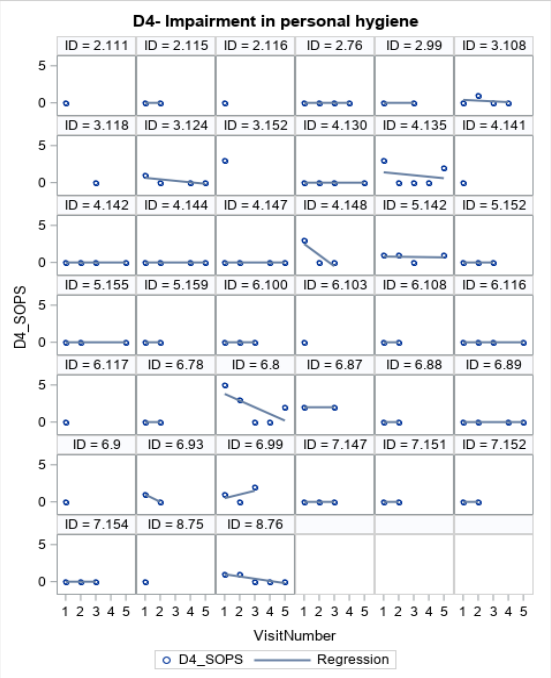
The figure represents change in D2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 36. Trouble with focus and Attention (D3) among non-converted



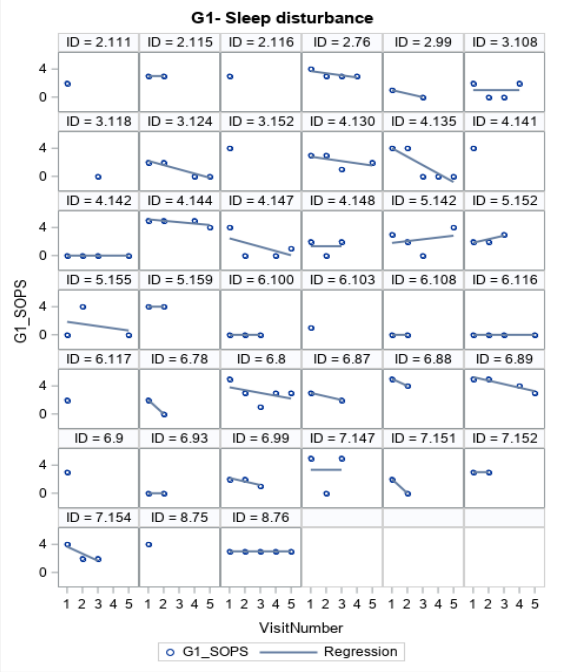
The figure represents change in D3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 37. Impairment in personal Hygiene (D4) among non-converted



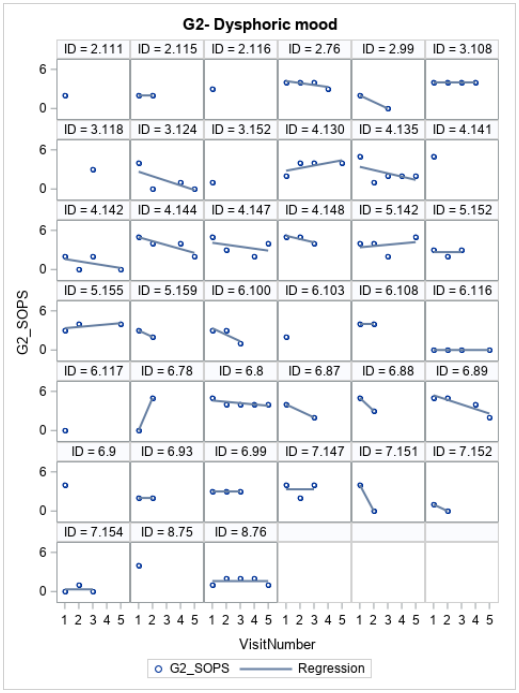
The figure represents change in D4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 38. Sleep disturbance (G1) among non-converted



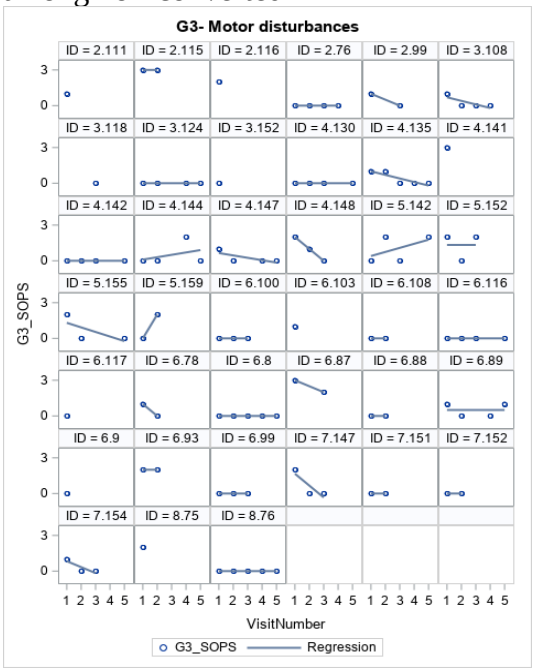
The figure represents change in G1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 39. Dysphoric mood (G2) among non-converted



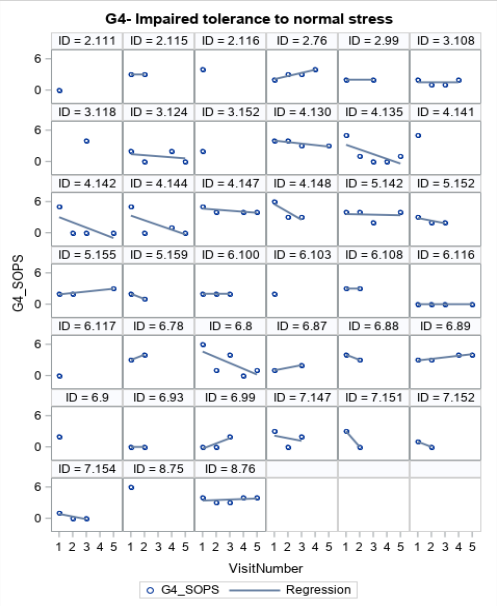
The figure represents change in G2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 40. Motor disturbances (G3) among non-converted



The figure represents change in G3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

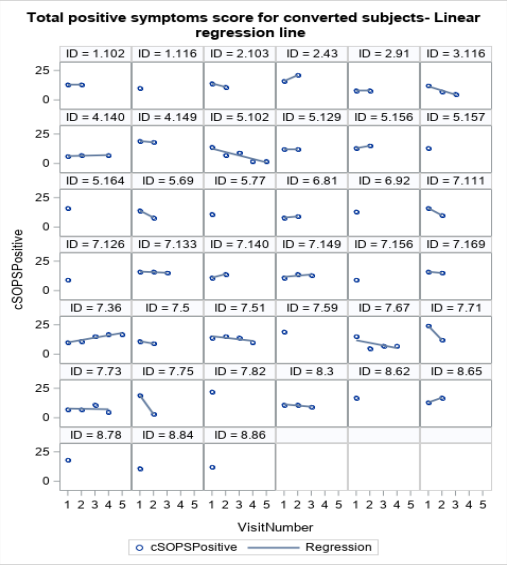
Figure 41. Impaired tolerance to normal stress (G4) among non-converted



The figure represents change in G4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

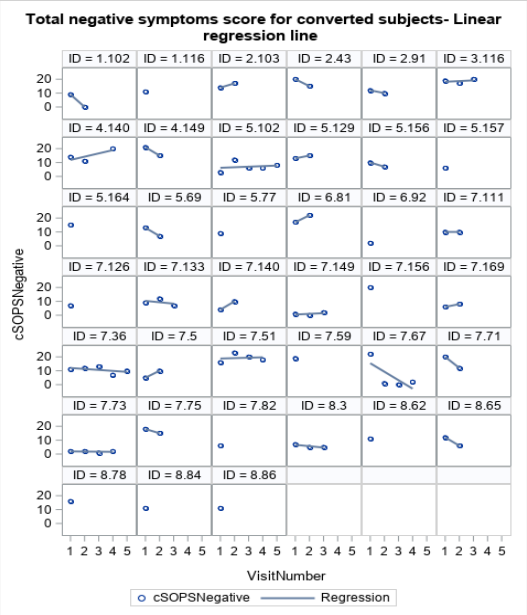
Figure 42-49. Individual changes in combined domain symptoms score by conversion status, over time in a sub-sample of youth from NAPLS study

Figure 42. Total positive symptoms score (cSOPSPositive) among converted



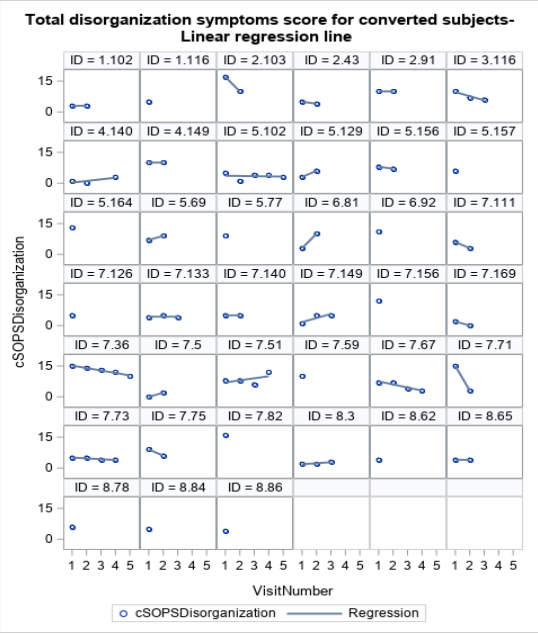
The figure represents change in combined positive symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 43. Total negative symptoms score (cSOPSNegative) among converted



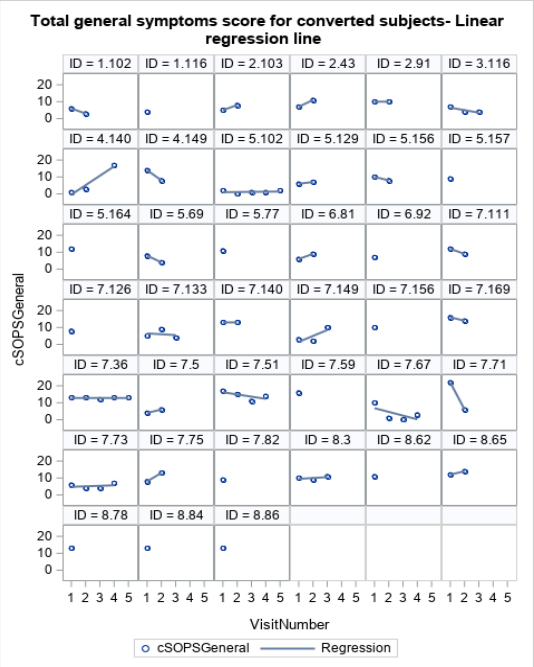
The figure represents change in combined negative symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 44. Total disorganization symptoms score (cSOPSDisorganization) among converted



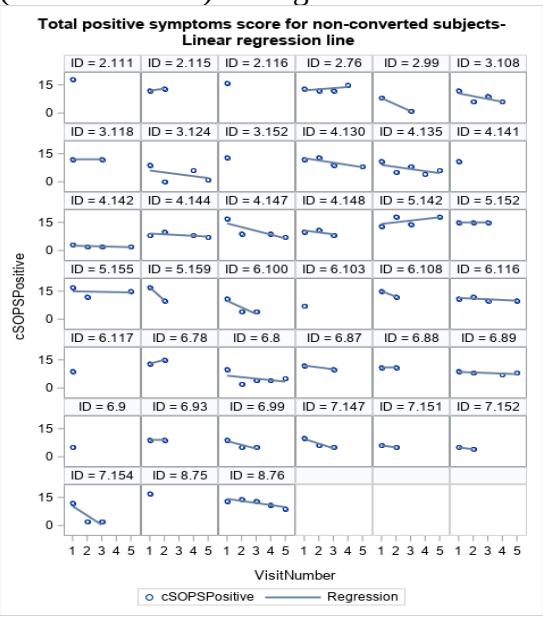
The figure represents change in combined disorganization symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 45. Total general symptoms score (cSOPSGeneral) among converted



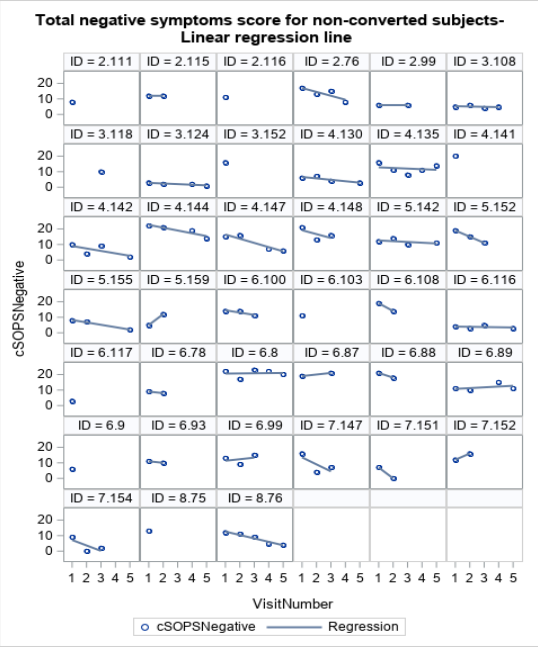
The figure represents change in combined general symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each converted subject

Figure 46. Total positive symptoms score (cSOPSPositive) among non-converted



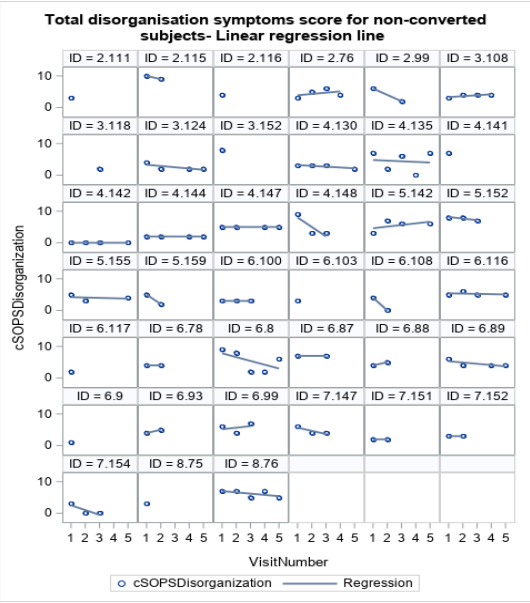
The figure represents change in combined positive symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 47. Total negative symptoms score (cSOPSNegative) among non-converted



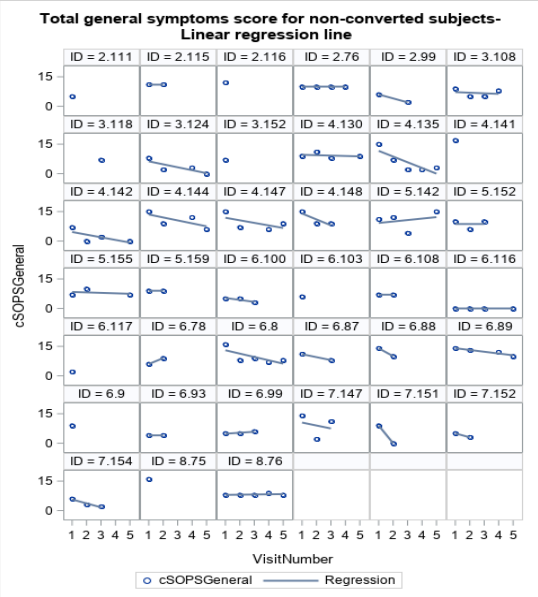
The figure represents change in combined negative symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 48. Total disorganization symptoms score (cSOPSDisorganization) among non-converted



The figure represents change in combined disorganization symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figure 49. Total general symptoms score (cSOPSGeneral) among non-converted



The figure represents change in combined general symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months) for each non-converted subject

Figures 50- 68. Inter-individual differences in each of the 19 prodromal symptoms by C4 concentration dichotomized around the mean (=2.5 ng/ml) and conversion status over time in the sub-sample of youth from NAPLS study

Figure 50. Unusual thought content/ delusional ideas (P1)

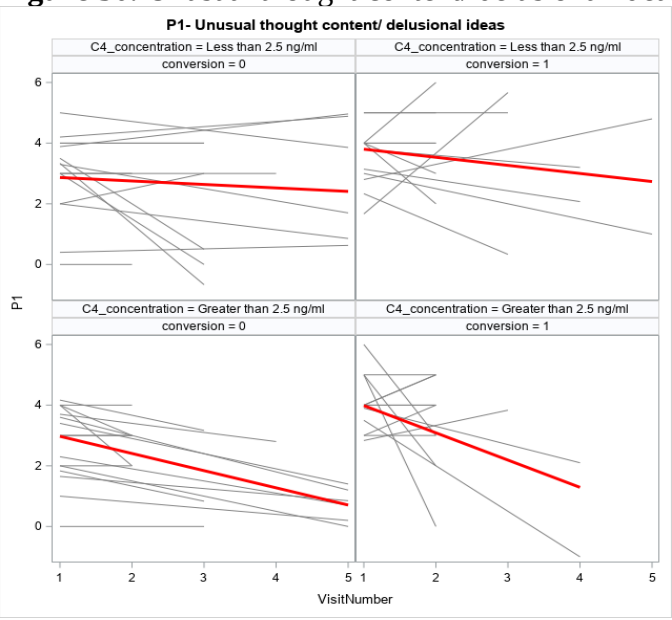


Figure represents change in P1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 51. Suspiciousness/ persecutory ideas (P2)

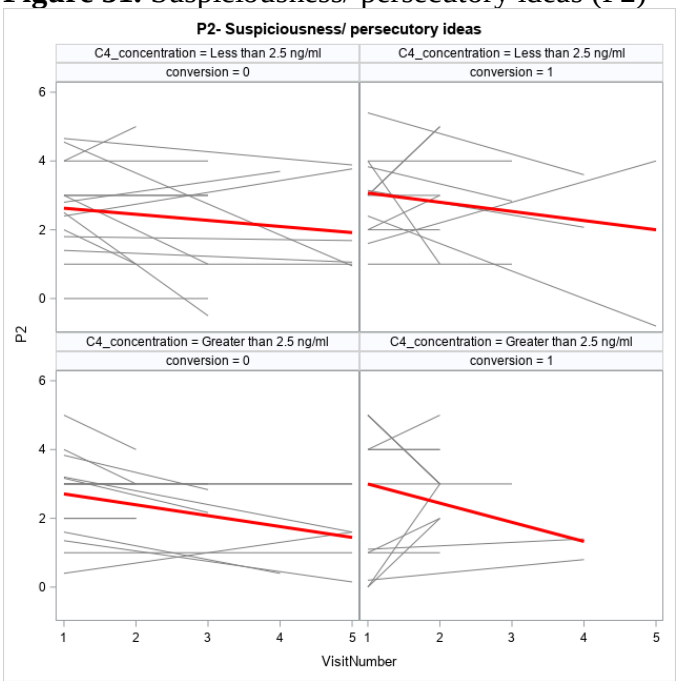


Figure represents change in P2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 52. Grandiose ideas (P3)

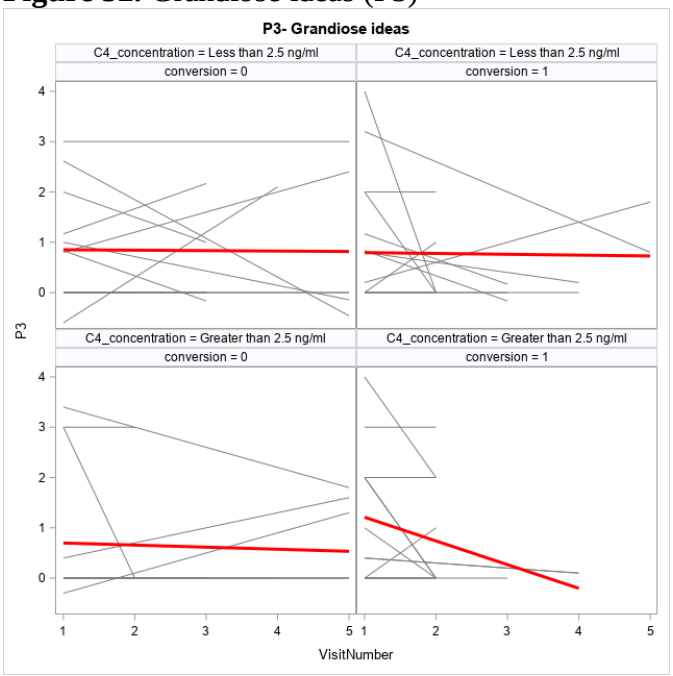


Figure represents change in P3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 53. Perceptual abnormalities/ hallucinations (P4)

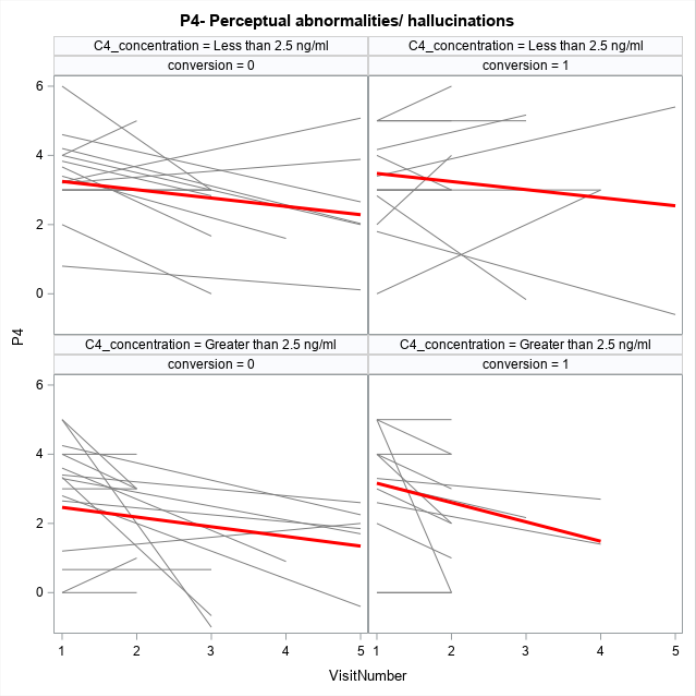


Figure represents change in P4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 54. Disorganized communication (P5)

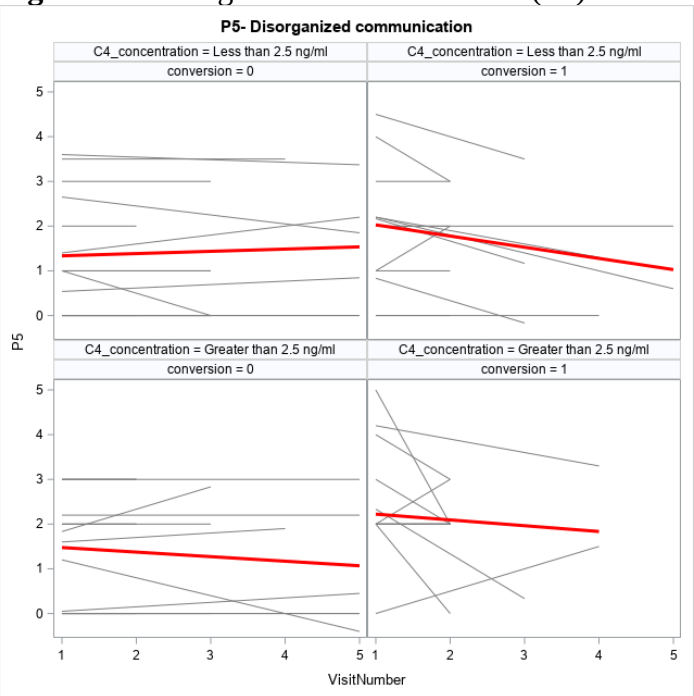


Figure represents change in P5 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 55. Social anhedonia (N1)

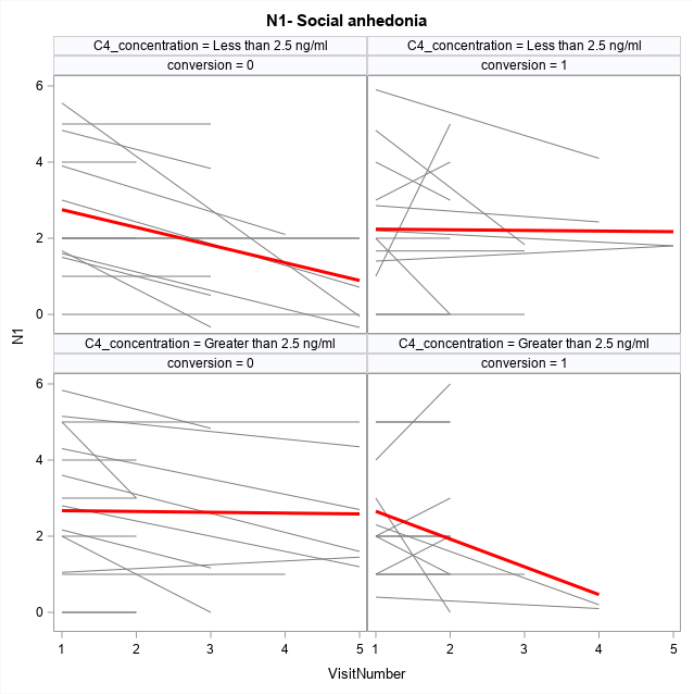


Figure represents change in N1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 56. Avolition (N2)

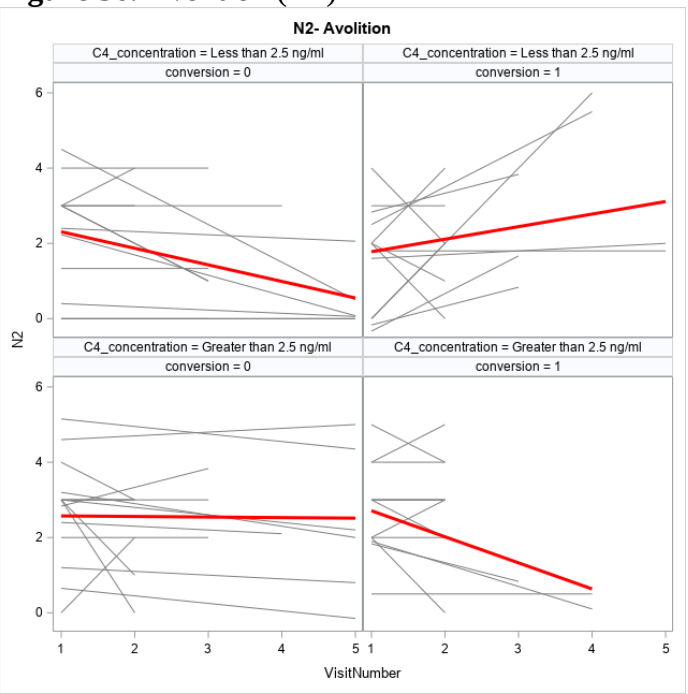


Figure represents change in N2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 57. Decreased expression of emotion (N3)

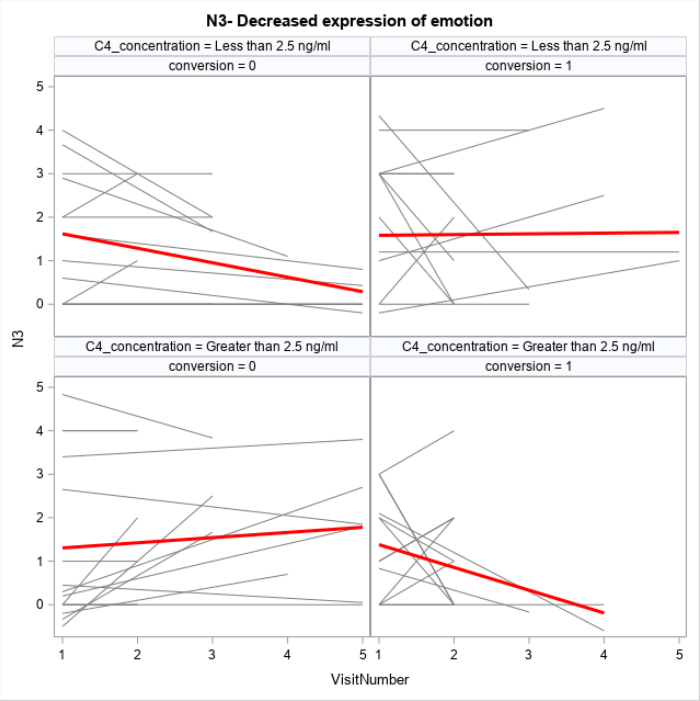


Figure represents change in N3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 58. Decreased expression of emotions and self (N4)

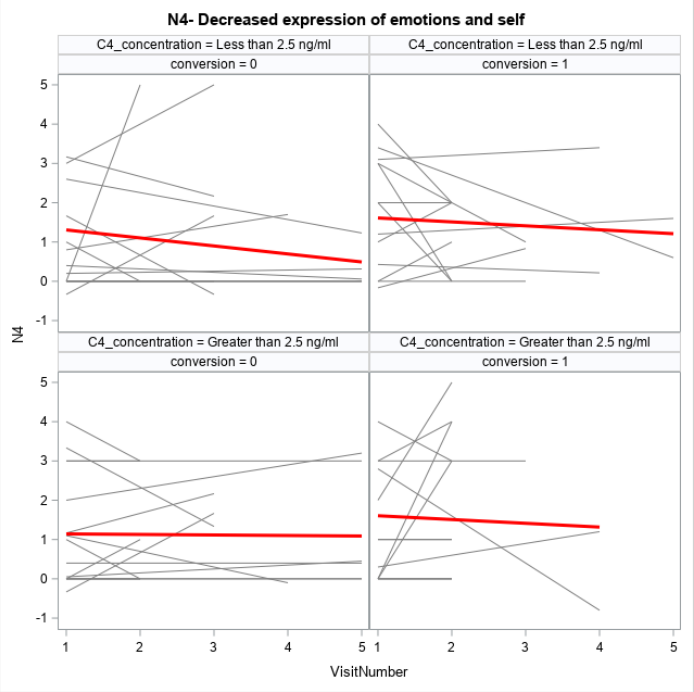


Figure represents change in N4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 59. Decreased ideational richness (N5)

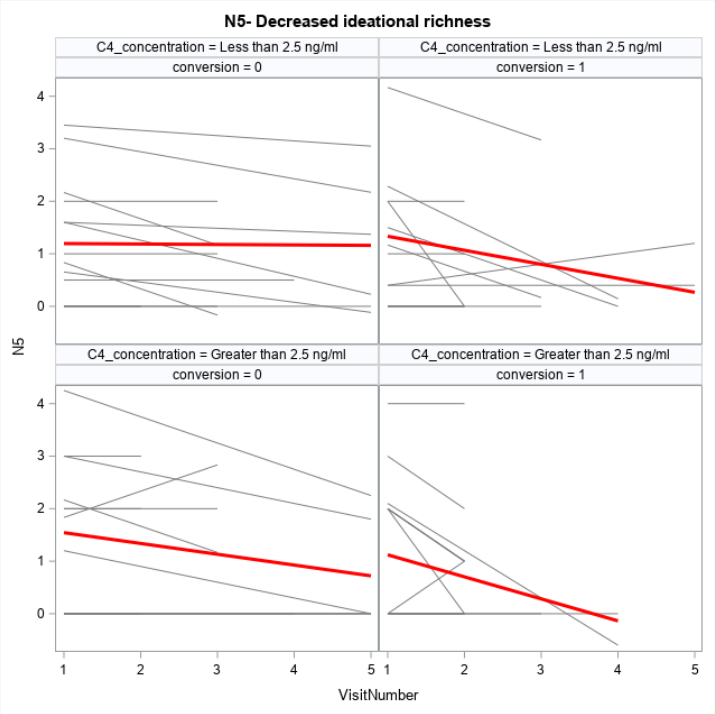


Figure represents change in N5 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 60. Occupational functioning (N6)

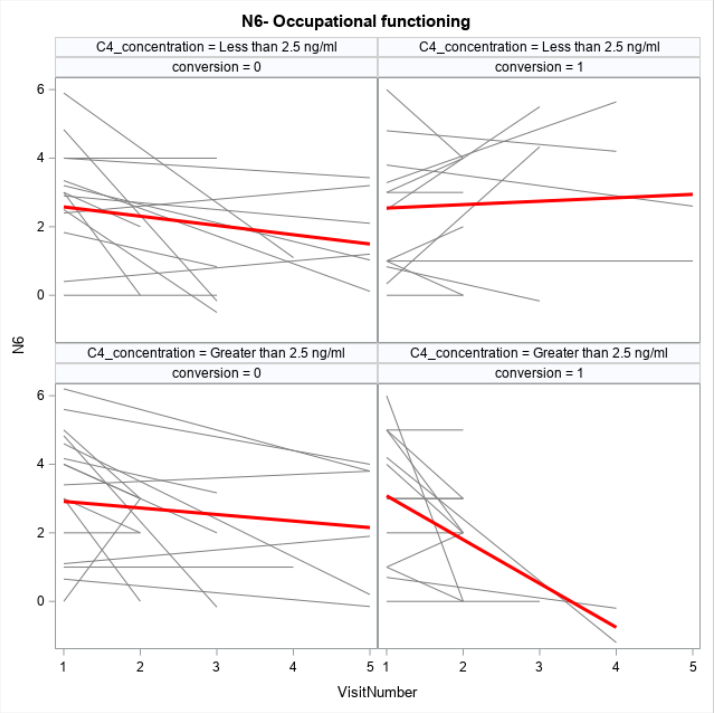


Figure represents change in N6 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 61. Odd behaviour or appearance (D1)

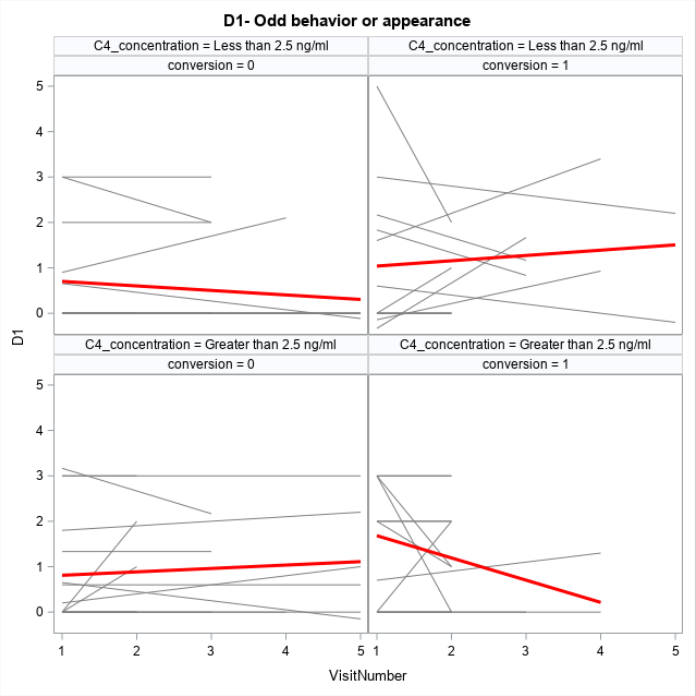


Figure represents change in D1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 62. Bizarre thinking

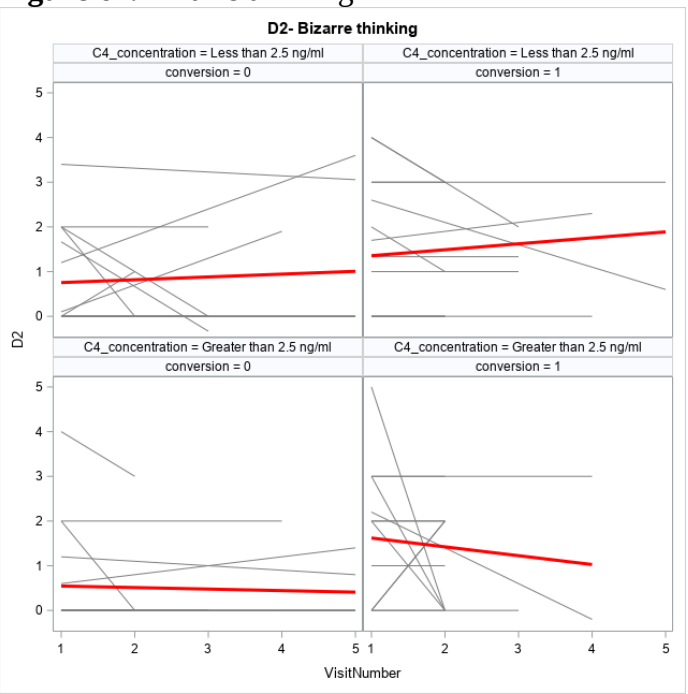


Figure represents change in D2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 63. Trouble with focus and attention (D3)

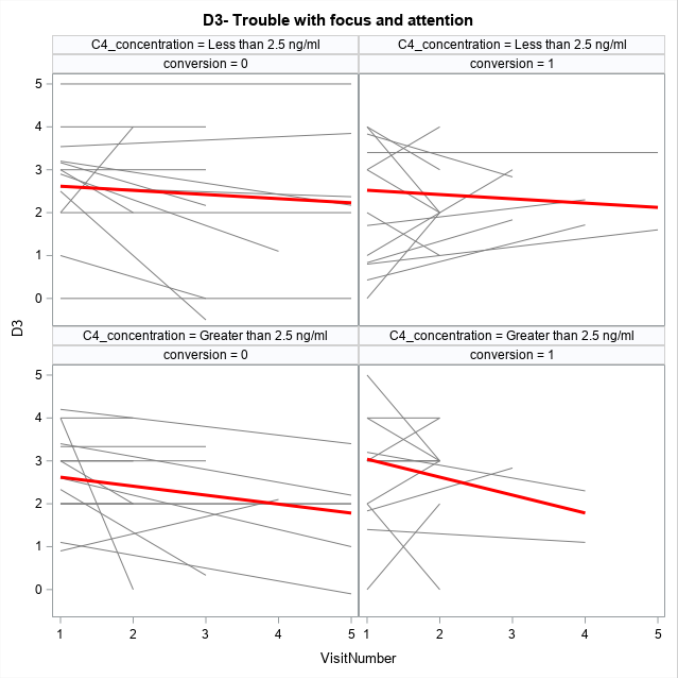


Figure represents change in D3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 64. Impairment in personal hygiene (D4)

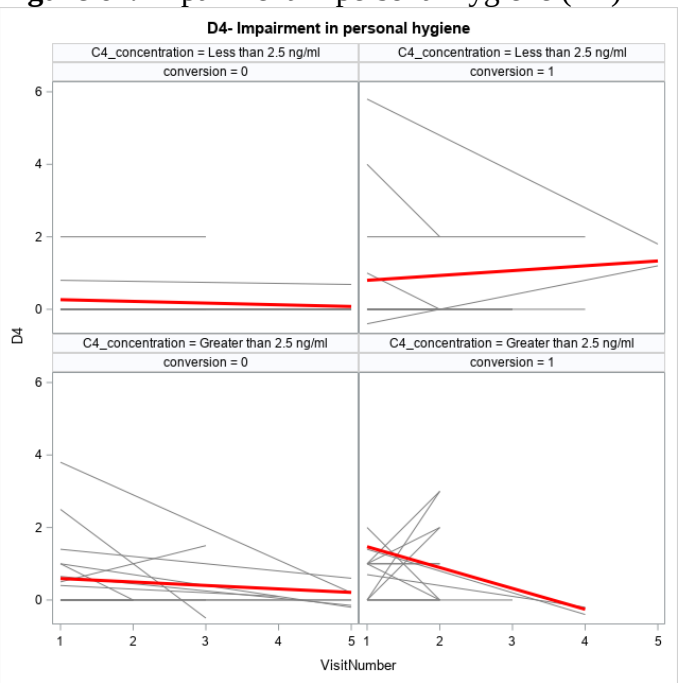


Figure represents change in D4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 65. Sleep disturbance

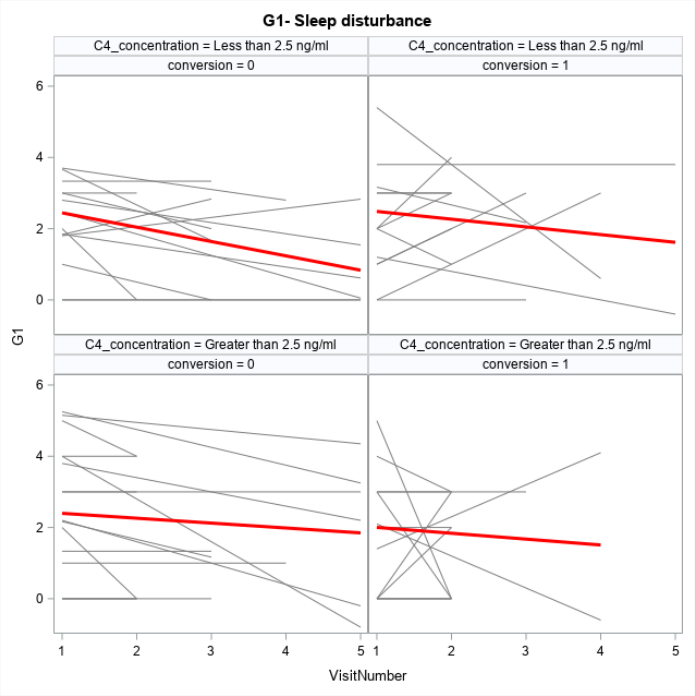


Figure represents change in G1 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 66. Dysphoric mood (G2)

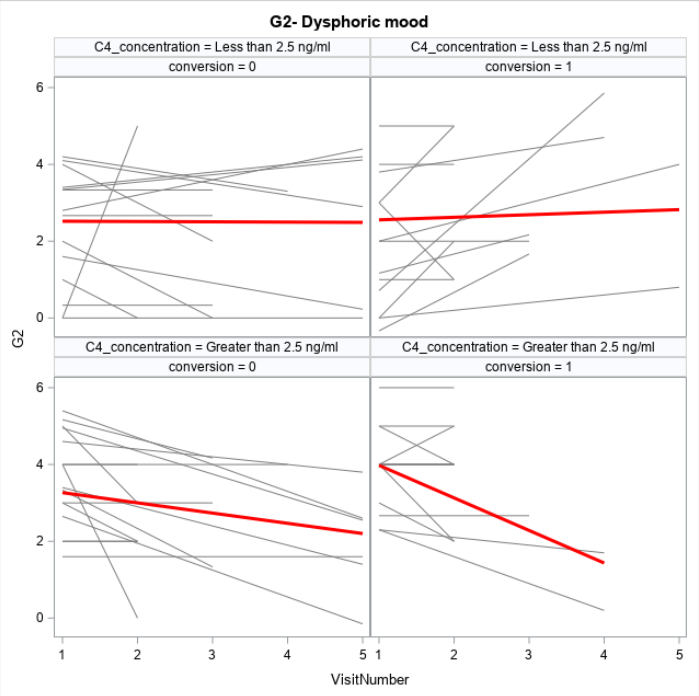


Figure represents change in G2 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 67. Motor disturbances

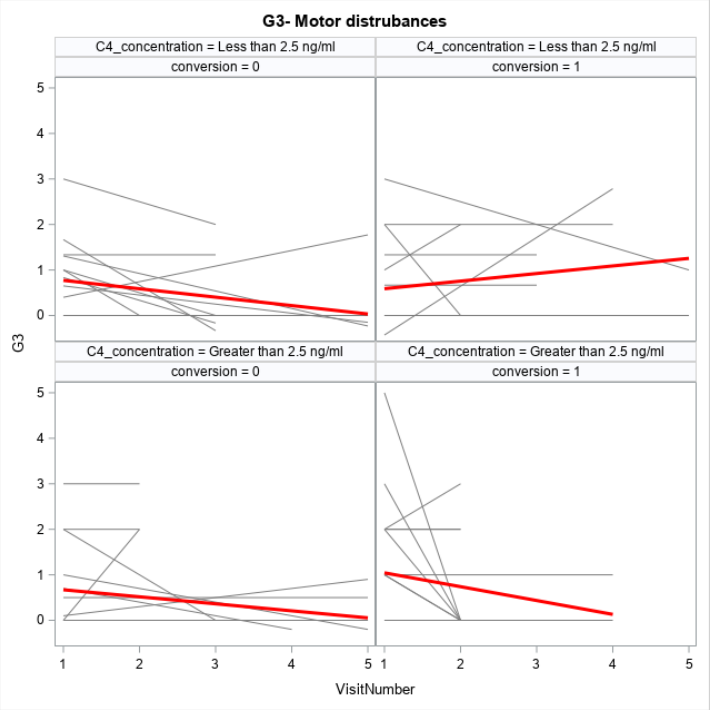


Figure represents change in G3 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 68. Impaired tolerance to normal stress

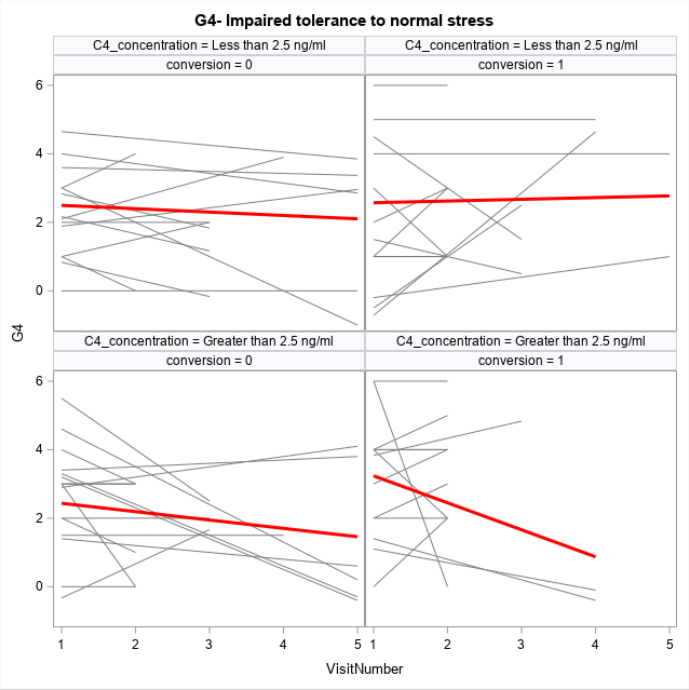


Figure represents change in G4 symptom over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figures 69- 72. Inter-individual differences in combined prodromal symptoms scores by C4 concentration dichotomized around the mean (=2.5 ng/ml) and conversion status over time in the sub-sample of youth from NAPLS study

Figure 69. Total positive symptoms score

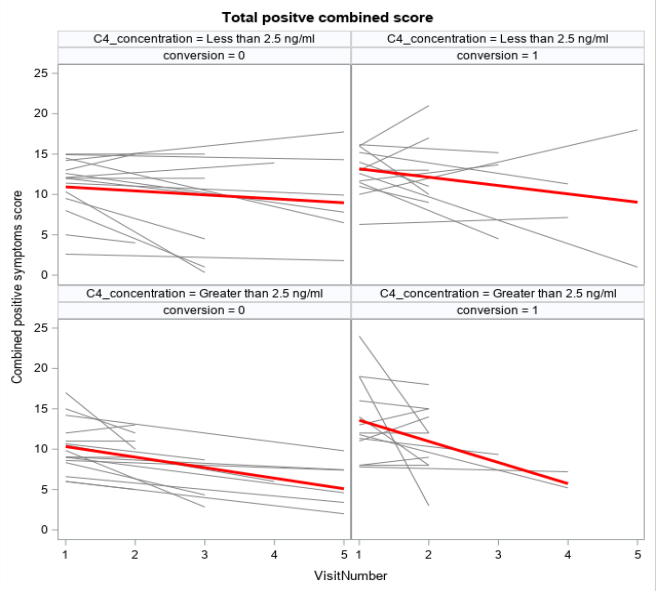


Figure represents change in total positive symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 70. Total negative symptoms score

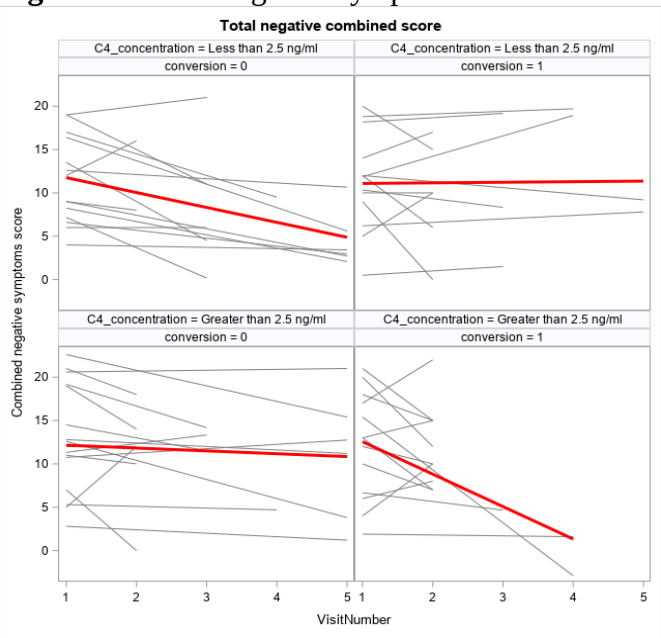


Figure represents change in total negative symptoms Score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 71. Total disorganization symptoms score

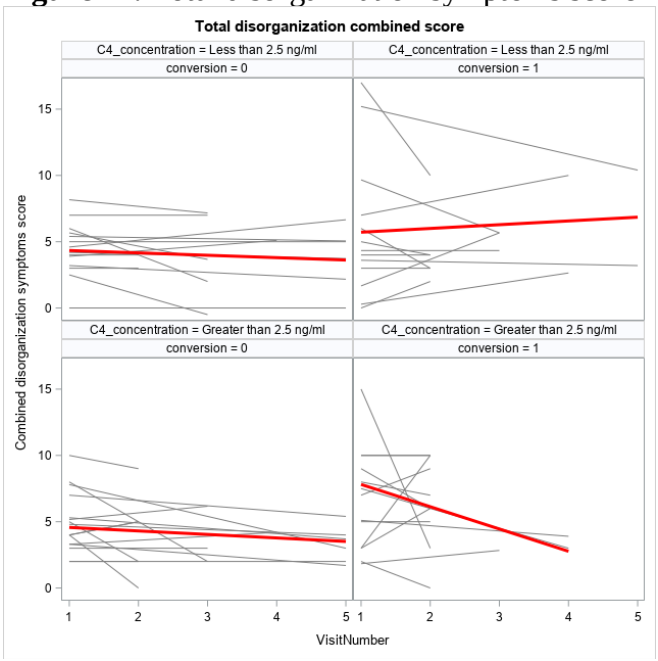


Figure represents change in total disorganization symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

Figure 72. Total general symptoms score

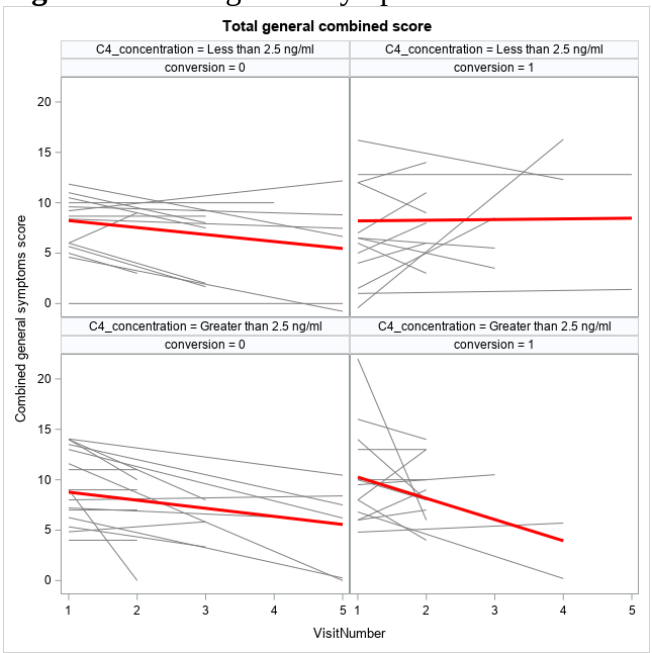


Figure represents change in total general symptoms score over time (where 1 to 5 represents visits at BL, 6 months, 12 months, 18 months and 24 months), grouped by conversion status and dichotomous C4 concentration

9. Appendices

Appendix 1. Complement component C4 concentration in saliva from 40 adolescents at CHR for Psychosis (Plate 1), NAPLS study, 2008-2015

Well ID	Well	Adjusted 450-570	Conc	Count	Mean	Std Dev	CV (%)
SPL1	A3	0.29	0.888	2	0.869	0.028	3.17
	A4	0.276	0.849				
SPL2	B3	0.421	1.268	2	1.244	0.035	2.82
	B4	0.404	1.219				
SPL3	C3	0.657	1.967	2	1.933	0.048	2.5
	C4	0.635	1.899				
SPL4	D3	0.785	2.366	2	2.337	0.041	1.74
	D4	0.767	2.308				
SPL5	E3	0.754	2.268	2	2.252	0.022	0.959
	E4	0.744	2.237				
SPL6	F3	0.209	0.65	2	0.634	0.022	3.45
	F4	0.199	0.619				
SPL7	G3	1.532	5.336	2	5.256	0.113	2.16
	G4	1.5	5.176				
SPL8	H3	0.493	1.479	2	1.43	0.068	4.78
	H4	0.46	1.382				
SPL9	A5	0.864	2.621	2	2.591	0.043	1.65
	A6	0.845	2.561				
SPL10	B5	0.267	0.823	2	0.825	0.003	0.403
	B6	0.269	0.827				
SPL11	C5	0.22	0.683	2	0.679	0.006	0.813
	C6	0.218	0.675				
SPL12	D5	1.232	3.969	2	3.971	0.002	0.0581
	D6	1.233	3.973				
SPL13	E5	0.277	0.853	2	0.848	0.007	0.807
	E6	0.274	0.843				
SPL14	F5	0.326	0.994	2	0.993	0	0.0413
	F6	0.326	0.993				
SPL15	G5	0.095	0.285	2	0.284	0.001	0.263
	G6	0.095	0.284				
SPL16	H5	0.623	1.865	2	1.826	0.055	3.01
	H6	0.597	1.787				
SPL17	A7	0.622	1.861	2	1.841	0.027	1.49
	A8	0.609	1.822				
SPL18	B7	1.55	5.429	2	5.369	0.086	1.6
	B8	1.527	5.308				
SPL19	C7	0.291	0.891	2	0.89	0.001	0.116

	C8	0.29	0.889				
SPL20	D7	0.391	1.183	2	1.185	0.002	0.138
	D8	0.392	1.186				
SPL21	E7	0.807	2.435	2	2.407	0.04	1.66
	E8	0.789	2.379				
SPL22	F7	1.068	3.334	2	3.247	0.123	3.8
	F8	1.02	3.16				
SPL23	G7	0.135	0.419	2	0.412	0.011	2.63
	G8	0.131	0.404				
SPL24	H7	0.942	2.886	2	2.847	0.056	1.97
	H8	0.919	2.807				
SPL25	A9	0.945	2.894	2	2.84	0.077	2.71
	A10	0.913	2.785				
SPL26	B9	2.583	16.103	2	15.257	1.196	7.84
	B10	2.498	14.411				
SPL27	C9	1.824	7.052	2	6.986	0.094	1.34
	C10	1.804	6.919				
SPL28	D9	0.341	1.037	2	1.031	0.008	0.755
	D10	0.337	1.026				
SPL29	E9	0.817	2.467	2	2.451	0.022	0.904
	E10	0.807	2.436				
SPL30	F9	0.824	2.492	2	2.493	0.002	0.0829
	F10	0.825	2.495				
SPL31	G9	0.749	2.252	2	2.225	0.038	1.69
	G10	0.732	2.198				
SPL32	H9	2.32	11.681	2	11.407	0.388	3.4
	H10	2.277	11.133				
SPL33	A11	0.038	0.051	2	0.066	0.021	32.1
	A12	0.044	0.081				
SPL34	B11	0.209	0.649	2	0.661	0.017	2.58
	B12	0.217	0.673				
SPL35	C11	1.303	4.263	2	4.2	0.089	2.11
	C12	1.273	4.137				
SPL36	D11	0.495	1.485	2	1.54	0.077	5.02
	D12	0.532	1.594				
SPL37	E11	0.885	2.692	2	2.741	0.069	2.53
	E12	0.914	2.79				
SPL38	F11	0.634	1.897	2	1.863	0.048	2.57
	F12	0.612	1.829				
SPL39	G11	0.214	0.663	2	0.666	0.005	0.767
	G12	0.216	0.67				
SPL40	H11	0.061	0.158	2	0.149	0.012	8.39
	H12	0.057	0.14				

Appendix 2. Complement component C4 concentration in saliva from 38 adolescents at CHR for Psychosis (Plate 2), NAPLS study, 2008-2015

Well ID	Well	Adjusted 450-570	Conc	Count	Mean	Std Dev	CV (%)
SPL1	A3	0.285	1.725	2	1.688	0.052	3.09
	A4	0.275	1.651				
SPL2	B3	0.306	1.882	2	1.82	0.087	4.77
	B4	0.29	1.759				
SPL3	C3	0.532	3.701	2	3.738	0.053	1.41
	C4	0.541	3.775				
SPL4	D3	1.919	18.168	2	17.995	0.245	1.36
	D4	1.89	17.822				
SPL5	E3	0.109	0.525	2	0.485	0.057	11.8
	E4	0.095	0.444				
SPL6	F3	1.015	8.197	2	8.187	0.014	0.173
	F4	1.013	8.177				
SPL7	G3	0.908	7.138	2	7.024	0.16	2.28
	G4	0.884	6.911				
SPL8	H3	0.725	5.414	2	5.093	0.454	8.91
	H4	0.654	4.772				
SPL9	A5	0.947	7.522	2	7.661	0.196	2.56
	A6	0.975	7.8				
SPL10	B5	0.508	3.494	2	3.388	0.15	4.43
	B6	0.482	3.282				
SPL11	C5	0.712	5.29	2	5.375	0.121	2.25
	C6	0.73	5.461				
SPL12	D5	2.189	>21.000	0	?????	?????	?????
	D6	2.199	>21.000				
SPL13	E5	0	<0.000	1	0.097	?????	?????
	E6	0.029	0.097				
SPL14	F5	0.76	5.734	2	5.716	0.026	0.448
	F6	0.756	5.697				
SPL15	G5	0.592	4.219	2	4.394	0.249	5.66
	G6	0.632	4.57				
SPL16	H5	0.559	3.935	2	3.874	0.087	2.24
	H6	0.545	3.813				
SPL17	A7	1.268	10.802	2	11.235	0.613	5.46
	A8	1.349	11.669				
SPL18	B7	0.428	2.837	2	2.405	0.611	25.4
	B8	0.318	1.973				
SPL19	C7	1.493	13.249	2	12.98	0.379	2.92
	C8	1.445	12.712				
SPL20	D7	1.12	9.253	2	9.202	0.072	0.787
	D8	1.11	9.151				
SPL21	E7	1.138	9.445	2	9.305	0.197	2.11

	E8	1.111	9.166				
SPL22	F7	0.53	3.688	2	3.454	0.33	9.56
	F8	0.475	3.221				
SPL23	G7	0.762	5.755	2	4.913	1.191	24.2
	G8	0.575	4.07				
SPL24	H7	0.766	5.79	2	5.666	0.176	3.1
	H8	0.739	5.542				
SPL25	A9	0.562	3.958	2	3.91	0.068	1.73
	A10	0.551	3.862				
SPL26	B9	1.501	13.333	2	13.629	0.419	3.07
	B10	1.554	13.925				
SPL27	C9	0.371	2.383	2	2.467	0.118	4.78
	C10	0.392	2.55				
SPL28	D9	0.099	0.468	2	0.463	0.008	1.62
	D10	0.097	0.458				
SPL29	E9	1.483	13.131	2	13.284	0.216	1.63
	E10	1.51	13.437				
SPL30	F9	0.599	4.279	2	4.139	0.198	4.79
	F10	0.567	3.999				
SPL31	G9	0.565	3.988	2	3.826	0.229	5.98
	G10	0.528	3.665				
SPL32	H9	0.254	1.495	2	1.424	0.1	7.04
	H10	0.234	1.353				
SPL33	A11	1.455	12.823	2	12.645	0.252	1.99
	A12	1.422	12.467				
SPL34	B11	0.512	3.527	2	3.488	0.055	1.59
	B12	0.502	3.449				
SPL35	C11	0.223	1.276	2	1.338	0.087	6.48
	C12	0.24	1.399				
SPL36	D11	1.373	11.925	2	11.253	0.95	8.44
	D12	1.247	10.582				
SPL37	E11	0.129	0.65	2	0.653	0.004	0.542
	E12	0.13	0.655				
SPL38	F11	0.248	1.456	2	1.423	0.047	3.27
	F12	0.239	1.39				
SPL39	G11	0.726	5.423	2	5.326	0.137	2.57
	G12	0.705	5.229				
SPL40	H11	0.822	6.311	2	5.899	0.583	9.88
	H12	0.733	5.487				

Appendix 3. Sensitivity analysis with weight in the model for conversion status associated with C4 concentration in the NAPLS study, 2008-2015

Model	OR	95% CI	p-value
Fully adjusted model[1]	0.92	0.80, 1.07	0.29

[1] The model was adjusted for sex, race, age (years), income > 40,000 and weight (in pounds)

Appendix 4. Frequency at Baseline of the number of clinical high-risk subjects witnessing each of the 19 symptoms on the Scale of Prodromal Symptoms (N= 78)

Symptom	Mean (SD)	Number (%) witnessing with severity>2
Positive Symptoms		
P1-Unusual thought content/ Delusional Ideas	0.81 (0.40)	63 (80.77)
P2-Suspiciousness/ Persecutory Ideas	0.64 (0.48)	50 (64.10)
P3-Grandiose ideas	0.15 (0.36)	12 (15.38)
P4-Perceptual Abnormalities/ Hallucinations	0.77 (0.42)	60 (76.92)
P5-Disorganized Communication	0.28 (0.46)	22 (28.21)
Negative Symptoms		
N1-Social anhedonia	0.42 (0.50)	32 (41.56)
N2-Avolition	0.51 (0.50)	39 (50.65)
N3-Decreased expression of emotion	0.30 (0.46)	23 (29.87)
N4-Decreased experience of emotions and self	0.30 (0.46)	23 (29.87)
N5-Decreased ideational richness	0.21 (0.41)	16 (20.78)
N6-Occupational functioning	0.61 (0.49)	47 (61.04)
Disorganization Symptoms		
D1-Odd behavior or appearance	0.22 (0.42)	17 (22.08)
D2-Bizarre thinking	0.17 (0.38)	13 (16.88)
D3-Trouble with focus and attention	0.65 (0.48)	50 (64.94)
D4-Impairment in personal hygiene	0.16 (0.37)	12 (15.58)
General Symptoms		
G1-Sleep disturbance	0.53 (0.50)	41 (53.25)
G2-Dysphoric mood	0.68 (0.47)	52 (67.53)
G3-Motor disturbances	0.08 (0.27)	6 (7.79)
G4-Impaired tolerance to normal stress	0.55 (0.50)	42 (54.55)