Open Journal of Psychiatry, 2015, 5, 78-112
Published Online January 2015 in SciRes. http://www.scirp.org/journal/ojpsych
http://dx.doi.org/10.4236/ojpsych.2015.51011

Biomarker Symptom Profiles for Schizophrenia and Schizoaffective Psychosis

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Received 2 January 2015; accepted 25 January 2015; published 28 January 2015

Abstract

Background: Neuroscience can assist clinical understanding and therapy by finding neurobiological markers for mental illness symptoms. Objectives: To quantify biomarkers for schizophrenia and schizoaffective disorder and relate these to discrete symptoms of psychosis. Methods: Within a case-control design with multiple exclusion criteria to exclude organic causes and confounding variables, 67 DSM IV-R diagnosed and 67 control participants from a defined hospital, clinic and community catchment area were investigated for candidate markers. Participants underwent protocol-based diagnostic-checking and symptom rating via Brief Psychiatric Rating Scale and Positive and Negative Syndrome Scale, functional-rating scales, biological sample-collection and sensory-processing assessment. Blood and urine samples were analysed for monoamine neurotransmitters, their metabolites, vitamin cofactors and intermediate-substances related to oxidative stress and metabolism of monoamines. Neurocognitive assessment of visual and auditory processing was conducted at both peripheral and central levels. Biomarkers were defined by Receiver Operating Curve (ROC) analysis. Spearman's analysis explored correlations between discrete symptoms and the biomarkers. Results: Receiver Operating Curve (ROC) analysis identified twenty-one biomarkers: elevated urinary dopamine, noradrenaline, adrenaline and hydroxy pyrroline-2-one as a marker of oxidative stress. Other biomarkers were deficits in vitamins D, B6 and folate, elevation of serum B12 and free serum copper to zinc ratio, along with deficits in dichotic listening, distance vision, visual and auditory speed of processing, visual and auditory working memory and six middle ear acoustic reflex parameters. Discrete symptoms such as delusions, hostility, suicidality and auditory hallucinations were biomarker-defined and symptom biomarker correlations assumed an understandable pattern in terms of the catecholamines and their relationship to biochemistry, brain function and disconnectivity. Conclusions: In the absence of a full diagnosis, biomarker-symptom-signatures inform psychiatry about the structure of psychosis and provide an understandable pattern that points in the direction of a new neurobiological system of

How to cite this paper: Fryar-Williams, S. and Strobel, J.E. (2015) Biomarker Symptom Profiles for Schizophrenia and Schizoaffective Psychosis. *Open Journal of Psychiatry*, **5**, 78-112. http://dx.doi.org/10.4236/ojpsych.2015.51011

symptom-formation and treatment.

Keywords

Biomarkers, Schizophrenia, Psychosis, Mental Illness, Symptom-Profiles

1. Introduction

The architecture of schizophrenia is still largely unknown and conventional categories for diagnosing schizophrenia and schizoaffective states are still based on descriptions of symptoms and behaviours [1]. While progress has been made regarding the underlying molecular biology and neuropathology of schizophrenia, characterization of discrete symptoms does not reflect underlying neurobiological mechanisms [2]. Schizophrenia and related psychotic conditions are increasingly viewed as complex, polygenic diseases involving overlap of hundreds of forms of functional pathology. Although the search for peripheral and central markers for schizophrenia has been underway for many years investigating monoamine, neuro-immune, inflammatory, neuroendocrine and neuroplasticity markers and others [3] [4], only marginal understanding of symptom-formation in psychosis has been gained and no integrated, causal framework for therapy has yet emerged [5]. At the same time, clinicians have recognized changing diagnoses across time and added comorbidity within their patients' conditions, to the extent that there has been fading faith in fuzzy categorical boundaries within descriptive classification systems [6].

In the clinical setting, presenting symptoms that fail to join with others to meet sufficient criteria for any firm classified diagnosis cause clinicians considerable stress. Clinicians walk a fine line between knowing what a single symptom such as hallucinations might mean in the longer term and the knowledge that the earlier schizophrenia is confirmed and treatment is able to begin, then the better is the outcome [7]. Such symptoms may sometimes subside and unnecessary cost, distress and stigma may accompany an inaccurate diagnosis [8]. For these reasons, biomarkers for discrete symptoms of serious mental illness are urgently required, to complement phenomenology and inform clinical management [9].

Schizophrenia and schizoaffective disorder are allied conditions within the clinical setting, with the DSM-IV-R classification system describing schizoaffective disorder in terms of a major mood disorder episode occurring concurrent with symptoms that meet characteristic symptoms for schizophrenia [10]. The Mental Health Biomarker Project (Fryar-Williams 2010-2014) sought to discover, investigate and quantify biochemical and neuro-physiological markers of schizophrenia and schizoaffective disorder across a number of domains. These included neurotransmitter synthesis and metabolism, oxidative stress, nutrition-related vitamin and mineral co-factors, visual and auditory information processing and middle ear acoustic reflexes. The selection of vitamin and mineral cofactors in the biochemical domain related to their theoretical background in remote and proximal biochemical pathways related to monoamine synthesis and their purported relationship with monoamine metabolism through cofactor deficiencies in folate and methylation cycles. Also, their proximity to trans-sulfuration pathways relates to protection against oxidative stress. The theoretical background for selection of biological markers in this study is presented in **Figure 1**. The theory behind these relationships has been described in the literature [11]-[15] and research initiatives in this area have been well summarised by Frankenburg F.R. (2007), [16]. However, vitamin and mineral cofactors for enzymes within these biochemical pathways have not been quantified as biomarkers for mental illness. Indole-catecholamines have been extensively investigated in many body fluids, in relationship to psychosis and schizophrenia and there have been contrasting findings [17]. Urinary hydroxyhemopyrroline-2-one (HPL), a metabolite reported in schizophrenia is considered an indicator of disturbed porphyrin synthesis and oxidative stress [18]. Vitamins and mineral cofactors and intermediate substances related to folate and methionine (one-carbon) cycles have theoretical potential to exert subtle, cumulative effects on neurotransmitter synthesis and metabolism [19]-[21]. These allied pathways are affected by vitamin B6, B12, red cell folate and plasma homocysteine levels [22]-[25], serum copper [26], serum ceruloplasmin [27], red cell zinc [28], serum histamine [29] and serum methyltetrahydrofolate reductase (MTHFR 677 C- > T) gene polymorphism [30], whilst vitamin D has a proven epidemiological link with schizophrenia [31].

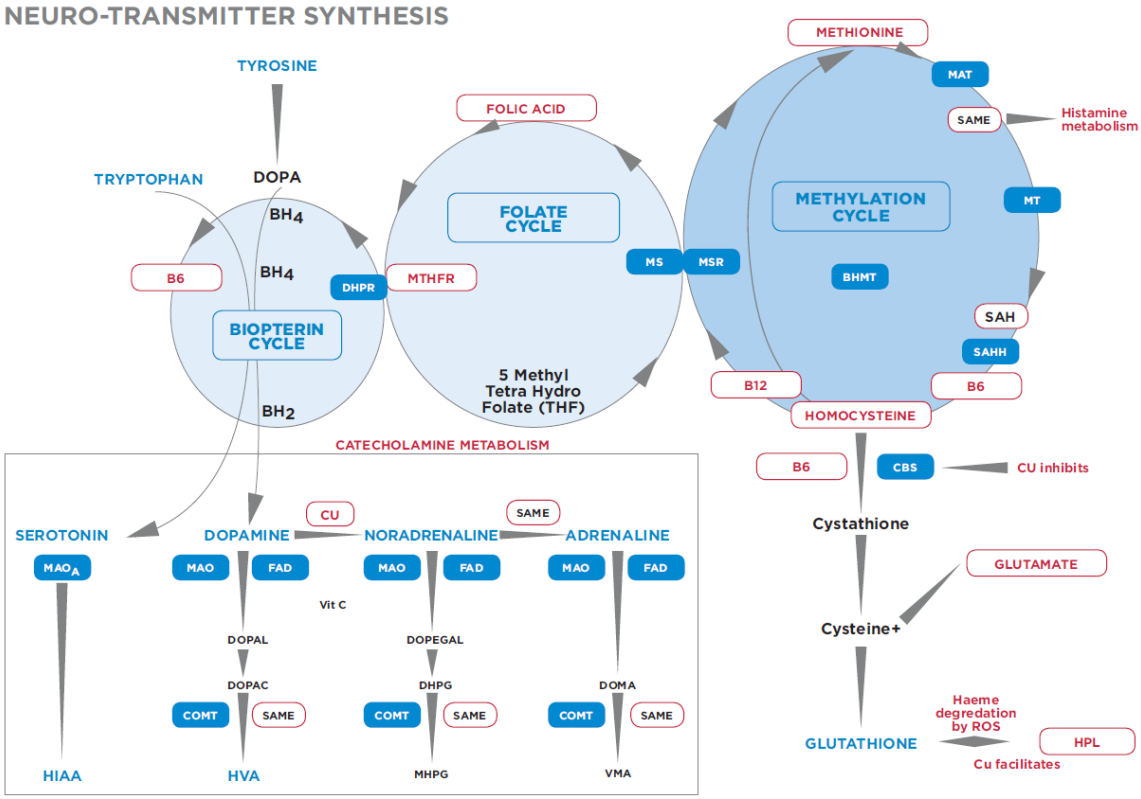

Figure 1. Theoretical background for selection of biological markers. MTHFR: Methylenetetrahydrofolate reductase, MAT: Methionine adenosyltransferase, SAMe: S-Adenosylmethionine, MT: Methyltransferase, SAH: S-Adenosylhomocysteine, SAHH: S-Adenosylhomocysteine-hydrolase, CBS: Cystathione Beta Synthetase, BHMT: Betainehomocysteine methyltrasferase, DMG: Dimethylglycine, TMG: Trimethylglycine, MSR: Methionine sulphoxide reductase, MS: Methioninesynthase, IAA: 5-hydroxyondolacetic acid, HVA: homovanillic acid, MAO: monoamineoxidase. MHMA: 3-methoxy-4-hydroxymandelic acid; VMA: Vanillylmandelic acid, FAD: Flavine adenine dinucleotide. MHPG: 4-hydroxy-3-methoxyphenylglycol, COMT: catec-hol-o-methyl-transferase. DOPAL: 3,4-dihydroxyphenylacetaldehyde, DOPAC: 3,4-dihydroxyphenylacetic acid, DOPEGAL: 3, 4-dihydroxyphenylglycolaldeyde, DOMA: 3,4-dihydroxymandelic acid.

2. Methods

2.1. Study Design

A case-control study design was used to clearly differentiate candidate markers between cases and controls. Multiple exclusion criteria (Section 2.3) were applied to case selection in order to strip psychosis in schizophrenia and schizoaffective disorder down to a functional core uninfluenced by organic causes such as substance abuse, head injury, sensory disability or medication variables that could unduly influence outcome measures.

2.2. Participants

This study was conducted between May 2010 and December 2014. Participants were enrolled between May 2010 and December 2013 at the Queen Elizabeth Hospital, Woodville, South Australia and at its satellite mental health clinics in the Western Adelaide community catchment area. All participants were informed of the goals, assessment procedures and funding of this study and provided written consent. Ethics permission for the study was obtained from the hospital ethics committee. Participants were from multi-ethnic backgrounds and the age-range was 18 to 60 years. A highly-characterized group of patients diagnosed with schizophrenia or schizoaffective disorder were compared with an age and gender matched group of controls, who had subclinical symptoms only. Similar psychotic symptoms occur in both schizophrenia and schizoaffective conditions [10] and these diagnoses occurred at a 1.2:1 ratio respectively in the ward and clinic population. Recruitment of patients with both of these allied conditions allowed sufficient numbers of patients with psychosis to be obtained

within the confines of the multiple exclusion criteria described in Section 2.3. It also gave scope for biomarker analysis of depressed mood that may co-occur in schizophrenia and depressed and manic symptoms that may co-occur in schizoaffective disorder.

In order to minimise setting variables participant assessments took place within a 4 day window. After case-note review, a standard interview protocol collected demographic information and information related to development, organic causes or sensory disability. Also recorded were height, weight and absence or presence of developmental difficulty or learning delay, medical co-morbidity, head-injury, family history of mental illness, use of glasses or hearing aids, in order to identify participants with peripheral sensory issues. Other information collected was time of onset of illness and duration of illness, social attachment durability and vision and hearing history.

All participants were rated using standard rating scales [32] [33]. These scales included the Brief Psychiatric Rating Scale (BPRS) and the Positive and Negative Syndrome Scale for schizophrenia (PANSS), which were amalgamated in the interest of reducing assessment time. Using this rating tool, each symptom was rated 1 to 7 intensity to give an overall symptom intensity rating (SIR), which was taken as a measure of symptom clinical severity. Hospital and clinic ratings were made by psychiatric trained registrars, who were blind to index laboratory and sensory processing test results, but not to patient status. Patient diagnostic checklist and symptom ratings for control participants were made by one psychiatric trained assessor who was not blind to the diagnostic status of the participant, since in the real-world clinic setting, many patients are unable to mask their condition due to residual symptoms of psychosis.

2.3. Patient Recruitment and Sample Selection

Non-detained ward patients in partial remission but with residual symptoms of psychosis, were recruited and assessed in the expected last week of their admission, by which time they were sufficiently recovered to give informed consent. Other patients were evaluated in satellite psychiatric outpatient clinics and the nearby research institute setting. A total of 82 symptomatic participants (cases) were recruited and completed assessment. Early statistical analysis of confounders required that 15 participants on SSRI or SNRI antidepressant medication be excluded from the study, due to the masking effect of SSRI and SNRI medication on catecholamine levels, sensory-processing and middle-ear outcome measurements. Therefore the number of patients in the final statistical analysis was 67.

Patients were included in the study if they received a diagnosis of schizophrenia or schizoaffective disorder made by a consultant psychiatrist in the ward or satellite psychiatric outpatient department, according to the DSM-IV-R classification criteria [10]. Diagnoses were checked against a DSM-IV-R symptom checklist, at the recruitment stage, to confirm that a correct diagnosis had been made. Ward patients were recruited within a week of their expected discharge back into the community so that severity bias between patients and community based controls was minimized. Ward patients were recruited and assessed in the ward setting and nearby research institute and community patients were recruited directly and by phone, from the satellite clinics they attended.

The multiple exclusion criteria applied to patient selection are now described: clinical appraisal of a patient's judgment capacity and orientation in time, place and person was undertaken during recruitment, in order to confirm capacity to consent and exclude delirium. Informal examination to exclude ocular muscle dysfunction, hand, forearm and shoulder dysfunction was also conducted at this stage, in order to ensure that a patient was free to proceed to neuro-physiological tests in the absence of rigidity, dyskinesia, tremor or postural instability as a result of extra-pyramidal side-effects from antipsychotic medication [34]. Persons medicated with Clozapine, Olanzapine, anti-histamines, or vitamin therapy were excluded, since these variables had potential to confound biochemical results for histamine. Participants taking antipsychotic-agents with lesser effect on histamine receptors (Zuclopenthixol, Modecate, Amisulpride (Solian) and Risperidone) were however included. The only exception was seven patients on Quetiapine. Mood stabilisers were allowed and antipsychotic medication remained stable during the assessment period. Persons with active or unremitted use of alcohol or other substance abuse were excluded, since this can confound neurotransmitter results. Persons with upper respiratory tract infections, intellectual, visual or auditory disability or clinically documented or descriptive history of hospitalized head injury with unconsciousness, were also excluded in order to ensure that middle ear congestion, known sensory disability or organic cerebral damage did not confound results. It was not possible to control for smoking to have any chance of patient recruitment.

2.4. Control Recruitment and Sample Selection

A total of 72 control participants were recruited with the assistance of the Population Research and Outcomes Studies (PROS) Unit of the University of Adelaide. These participants were volunteers from the same catchment area as patients affiliated with the Queen Elizabeth Hospital and the North West Adelaide Health Study (NWAHS). Using the same exclusion protocol as for patients, these participants were age-stratified and randomly recruited by phone over the same assessment period as patients. None of the controls reached symptom threshold for schizophrenia or any DSM-diagnosable mental illness, but were rated for reported and observed subclinical symptoms by a psychiatric trained assessor who was not blind to their asymptomatic status, but was blind to biological test results. Two younger controls required for younger age matching, were recruited from a local surf life-saving club within the same NWAHS catchment area. Five control participants were excluded due to their failure to meet exclusion criteria on assessment. The control sample used for the final analysis was drawn from the same catchment area as the patients and final sample consisted 67 controls.

2.5. Biochemical Assays and Specimen Collection

Laboratory tests used to assay biochemical markers in this study were commercially-available blood and urine samples, collected from all participants. Collection methods are documented in **Table 1**.

All biochemical testing was conducted by independent laboratories that were blind to participants' case or control status and all participant symptom raters and neurophysiological assessors were blind to laboratory results. Baseline fasting spot-urine samples were collected for biochemical assays of dopamine, noradrenaline and adrenaline as well as their metabolites homovanillic acid (HVA) and methoxy-hydroxymandelic acid (MHMA) and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA). As part of this analysis, urinary creatinine was determined as a urine concentration standard and this standard was also used for urinary hydroxyhemopyrroline-2-one (HPL). In order to ensure that urine neurotransmitter levels were not affected by hypothalamic pituitary axis (HPA) activation, due to the procedure of blood-taking, urine collection preceded blood-taking, by a minimum of two hours. Fasting blood was also collected for vitamins and mineral cofactors.

2.6. Sensory Processing Assessments

Reverse digit span working memory and spatial working memory deficits have a known association with schizophrenia [35] [36]. After determining visual and hearing acuity all participants were assessed for selected sensory and cognitive variables related to auditory and visual processing. Assessments were conducted in auditory and visual domains, at a time separated from both blood and urine collection and within 4 days of such biological sample-collection. Apart from the prerequisite audiogram assessment, equipment used is compact, carried in an attache case and the assessment time is forty five minutes (**Image 1** and **Image 2**).

Image 1. Sensory processing briefcase equipment (used with permission).

Image 2. Sensory processing speed assessment.

Where applicable, visual assessment was conducted using the participant's usual glasses and alternate-cover-test was conducted prior to visual testing, to exclude visual fixation disparity (phoria or tropia) as a potentially confounding variable [37]. Visual assessments used patients own glasses where appropriate and included near and distance visual acuity, visual attention span, speed and accuracy of visual performance as outlined in **Table 2**.

Auditory assessments were conducted in a quiet room (ambient noise level 20 dB) and preceded by examination of the external auditory meatus to exclude obvious pathology or sebum obstruction. Audiometric examination was conducted using the MAICO Audiogram MA 40 [38], at 250 Hz to 4000 Hz to determine hearing deficits (defined as air-bone conduction gaps > 10 Hz and/or sensory threshold abnormalities > 500 - 1000 Hz) and laterality differences. Auditory processing outcome measures were of acuity, attention and threshold speed and accuracy of auditory processing (**Table 3**).

In an effort to investigate pre-attentive deficits relating to acoustic reflexes and their relationship to central auditory-processing deficits or delays, specific measures were obtained for ear canal volume at threshold auditory response, peak middle ear pressure at threshold auditory response and gradient of the middle ear pressure elevation, using the Tymp GSI 38 by VIASYS Healthcare [39]. These measures were undertaken to quantify the tympanic muscle activating reflex response to sound entering the ear, along with the strength and duration of the sound dampening stapes muscle, which were surmised to be related to brain stimulus activation and inhibition, respectively. These two sequentially activated muscles regulate middle ear sound-conduction, with sound conductance by the tympanic and stapes muscle, followed by a sound dampening protective response as the handle of the stapes bone is finally applied to the oval window of the cochlea. In a novel approach, the strength and timing of the tympanic and stapes reflex responses were directly measured from the graph read-out of the machine, in order to ascertain whether any alacrity or delay of these sound-conducting and gating mechanisms might be associated with stimulus distortion in psychosis. Where repeated testing was necessary, an interval of 30 seconds or more was allowed between trials, in order to prevent error from muscle fatigue. Middle ear acoustic reflex assessment methods are documented in **Table 4**.

3. Data Analysis and Results

Data analysis on a sample consisting of 67 cases and 67 controls, within which there were 30 female cases with a mean age of 42 years and 37 males with a mean age of 39. In the control sample there were 34 females with a mean age of 45 and 33 males with a mean age of 46. For the total participant sample females totalled 64 and males totalled 70. The mean age for cases was 41 years and the mean age for controls was 46 years, matched on average to ±3 years. Variable distributions were mapped and quantile functions calculated using EasyFit software [40]. The sample was initially analysed as total cases versus total controls and no separate analysis by age and sex was conducted due to sample size. Basic demographic data relating to the participants and the

Table 1. Summary of biochemistry tests and methods with literature citations.

Test	Method, analyser, reagents	Laboratory/reference
Neurotransmitters		
Biogenic amines: dopamine, noradrenaline and adrenaline,	Spot-baseline (fasting) urinary neurotransmitter testing (second void morning), snap-frozen to minus 30 degrees and analysed by mass spectrometry, using nanomols per millimol of urinary creatinine as a standard.	SA Pathology, Adelaide, South Australia. Whiting MJ. 2009. Simultaneous measurement of urine metanephrines and catecholamines by liquid chromatography with tandem mass spectrometric detection. *Annals of Clinical Biochemistry*, **46**, 129-136.
Creatinine	Spot urine specimen from the same void as biogenic amines, expressed in millimols per Litre.	SA Pathology, Adelaide SA.
Oxidative stress: Urinary hydroxyhemopyrroline-2-one		
Urinary hydroxyhemopyrroline-2-one (HPL)	Spot urine (second void morning) in ascorbic acid, snap frozen (-30C) and light-protected. Colorimetric method at 540nm, following solvent extraction and reaction with Erich's reagent. micrograms per decilitre.	Applied Analytical Laboratories, 8/26 Nestor Drive, Meadowbrook, Queensland, Australia. +61 7 3133 1615.
Nutrition-Biochemistry		
Vitamin D (25-OH)	Diasorin Liason assay kit. for use on the Liaison platform nmol/L.	Clinpath Laboratories, 19 Fullarton Rd, Kent Town. South Australia 5067+61 8 8366 2000.
Serum total Vitamin B12	Competitive Electrochemiluminescent Immunoassay. Roche Modular E 170. Automated Immunoassay Analyser, using Roche Vitamin B12 Reagent (pmol/L).	Clinpath Laboratories. As above.
Red Cell Folate	Competitive Electrochemiluminescent Protein Binding Assay, using Roche Modular E 170, using Roche Folate Red Blood Cell (RBC) Reagent and Roche Folate RBC Haemolysing Reagent. on Automated Immunoassay Analyser (nmol/L).	Clinpath Laboratories. As above.
Vitamin B6 (Pyridoxal-5'-phosphate coenzyme form)	Whole blood High Pressure Liquid Chromatography with fluorescent detection. Chromsystems Vitamin B6 in Whole Blood High pressure Liqid Chromatography Reagent Kit. Waters Alliance 2695 Separations Module. Waters 474 Fluorescence Detector (nmol/L).	Sullivan Nicolaides Pathology 143 Whitmore St, Taringa. Queensland 4068. Australia. +61 7 337 8666
Serum Copper	Flame Atomic Absorption Spectrophotometry. Varian AA-240FS (umol/L).	Douglass Hanly Moir Pathology 14 Griffnock Avenue, Macquarie Park. New South Wales 2113. +61 2 9855 5222.
Red Cell Zinc	Inductively coupled plasma mass spectroscopy (ICP-MS), using 6% n-Butanol reagent and Agileny ICP-MS 7500ce analyser (umol/L).	Sullivan Nicolaides Pathology. As above.
Serum Ceruloplasmin	Immunoturbidimetric method, using 6K91-30 Multignet Caeruloplamin Kit and Abbott Architect ci16000 analyser (g/L).	Douglass Hanly Moir Pathology. As above.
Percentage Free Copper/Red Cell Zinc	Percentage of free copper in the serum calculated by an equation based on the molecular and atomic weights of ceruloplasmin and copper (one ceruloplasmin molecule binds to six copper atoms). The ratio of the percentage free copper to red cell zinc was calculated as "percentage free copper"/"Red cell zinc umol/L".	Calculated by authors
Intermediate substrates and enzymes		
MTHFR Ala222Val (C677T) methyl tetrahydrofolate reductase polymorphism	Real time PCR analysis Roche Diagnostics Light-Cycler 480 kit. Using TecnoBiol reagents, Sigma probes and primers on Roche LC480 analyser.	Douglass Hanly Moir Pathology. As above.
Plasma homocysteine	Ice transported EDTA sample. Competitive Chemiluminescent Immunoassay, using Seimens Homocysteine reagent on Seimens Advia centaur Automated Immunoassay (umol/L).	SA Pathology. +61 8 8222 3000 As above.
Serum histamine	Beckman Coulter Radio Immunoassay, using Beckman Coulter R.I.A. Kit on Perkin Elmer Wizard 1470 Automated Gamma Counter (umol/L).	Sullivan and Nicolaides. As above.

Table 2. Summary of visual assessment methods with literature citations.

Visual assessments	Method	Reference
Near vision acuity test	Sussex Vision test of near vision. Near vision test card SNT-3000-L, 2009-2011.	Sussex Vision test of near vision. Near vision test card SNT-3000-L, 2009-2011. Sussex Vision International Ltd.) http://Zazzle.com.au/near_vision_testcard_visual_acuity_exam-137420023635816730. Sussex Vision International Ltd.
Visual (symbol) span	Increasing number of symbols are presented in a standardised order from left to right. Test score reported as the absolute number of visual symbols recalled in the correct order.	Visual symbol Span. A subset test of WMS-IV. Weschler Memory Scale, Fourth Edition. 2009. Pearson Inc. Keith RW. Pearson Clinical Assessment P.O. Box 599700 San Antonio, Texas 78259
Distance vision (Binocular distance vision acuity)	Right distance vision, then left distance vision, with 20 seconds inter-test interval.	The Snellen-Chart (Snellen 1862) H. Snellen, Probebuchstaben zur Bestimmung der Sehschärfe, Utrecht 1862.
Threshold visual speed of processing performance as a percentage of age (Expresses visual processing speed in terms of the visual processing system's relative age) Shortest interval of time a person can notice between the order of presentation of two optical stimuli. Speed of visual order processing increases with age. For adults between the range of 18 and 60 years, the normal range for visual speed of processing is 24 to 72 milliseconds). For adults between the range of 18 and 60 years, the normal range for visual speed of processing is 24 to 72 milliseconds.	Person tested sees two brief flashes of light randomly presented from left-to-right or right-to-left on multiple occasions, and must decide which light flash appeared first. The inter-stimulus time interval (ISI) between the flashes is shortened by computer algorithm, if the answer is correct, otherwise it is lengthened. A performance-age rating, is provided, configured against norms-for-age. Performance-age is subtracted from the individual's actual age and the result divided by the age of the test subject is multiplied by 100.	Brain Boy Universal Professional instrument (MediTECH 2010). Langer Acker 7. D-30900 Wedemark, Germany.

Table 3. Auditory assessment methods with literature citations.

Auditory assessment	Method	Reference
Reverse digit span (Measures auditory (verbal) working memory) Normal range is 6 to 7	With gaze aversion by listening participant and tester, digits are read in set sequence. The tested participant is asked to repeat them in reverse order. Reported as the absolute number of digits correctly recalled in reverse order.	Weschler D.: Wechsler Adult Intelligence Scale-III (WAIS-III), 1997. The Psychological Corporation. Pearson Clinical Assessment, P.O. Box 599700, San Antonio, Texas 78259.
Competing words performance for age as a percentage of age (Intra-cerebral dichotic listening performance for processing of auditory information) Normal ranges vary with age	A voice-over CD and earphones test ability to correctly identify both of two competing-words (CW), delivered separately to the right and left ears. Using this test's normative-for-age database, the difference between each test subject's expected and actual performance-for-age was calculated, and this was then divided by the actual age of the test subject, and multiplied by 100.	Keith RW: SCAN-3 test for adolescents and adults. *Pearson Clinical and Talent Assessment* 2009. Published by Pearson ISBN 0158910176.
Threshold speed of Auditory processing as a percentage of age (Speed of auditory processing systems relative to age) Speed of auditory processing reduces with age. For adults in the age range of 18 and 60 years, the normal range for auditory speed of processing is 46 to 72 milliseconds.	Person tested hears two clicks, randomly presented from right to left and left to right side, presented through headphones. By pressing a right or left button, a decision must be made from which side the dual-stimulus originates. If the answer is correct, the inter-stimulus interval between flashes (ISI) is shortened, otherwise it is lengthened. The auditory order threshold is the shortest ISI a person can correctly differentiate between two auditory impressions. A read-out of the threshold speed of auditory (order) processing is provided, along with a norm performance-age rating. Auditory speed of (order) processing performance as a percentage of age is calculated by subtracting the norm-for-age from the performance-age, divided by the age of the test subject, multiplied by 100.	The Brain Boy Universal Professional Instrument (MediTECH 2010). By MediTECH Electronic GmbH, Langer Acker 7, 30900 Wedemark, Germany. www.meditech.de

Table 4. Summary of middle-ear assessment methods with website links.

Middle ear assessments	Method	Reference
Ear canal volume at threshold auditory response. Normal range > 0.2 cm·s^3	The GSI 38 measures combined volume of the ear canal and the middle ear space. An ear canal volume greater than 0.2 cm^3 may indicate tympanic membrane perforation due to trauma or abnormal laxity of the tympanic membrane in response to acoustic pressure.	GSI 38 Grason-Stadler 2005-Auto Tymp. Graason-Stadler A division of VIASYS Health Care, 5225. Verona Rd, Building 2, Madison, WI 53711.
Peak middle ear pressure at threshold auditory response Normal range −150 daPa to + 100 daPa	The GSI 38 measures peak middle ear pressure achieved in response to tympanic membrane change associated with tympanic muscle contraction. This is tested over the range of +200 daPa to −400 daPa. Low pressures may accompany Eustachian tube dysfunction.	GSI 38 (Grason-Stadler 2005)
Gradient middle ear pressure at threshold auditory response. Normal range 60 - 150 daPa	The GSI 38 measures the middle ear pressure gradient, for which the normal range is 50 to 110 daPa. High gradients are indicative of middle ear effusion.	GSI 38 (Grason-Stadler 2005).
Percentage length of the base of the stapes reflex divided by the total duration of the reflex. (A measure of the strength of the stapes reflex during its maximal period of contraction) The term "threshold" refers to the first reflex response which typically occurs at a frequency of 500Hz in a decibel range of 90 to 110.	The GSI 38 traces the stapes reflex contraction to maximum amplitude of 8 millimetres, after which it traces a basal threshold formed for the maximum portion of the reflex. The length of this basal portion divided by the total duration of the reflex contraction was multiplied by 100.	GSI 38 (Grason-Stadler 2005).
Projected Stapes amplitude (Alternative measure of stapes contraction strength)	Direct measurement of stapes amplitude at the intersection of projected onset and offset contraction gradients.	GSI 38 (Grason-Stadler 2005).
Time-to-off-set of the stapes reflex contraction divided by the base length (a measure of any acoustic-reflex offset, advance or delay).	Threshold base directly measured and compared with the total duration of the stapes reflex, from initiation to time of offset.	GSI 38 (Grason-Stadler 2005)

biomarker variables is provided in **Table 5** and **Table S2**, respectively. Statistical analysis was conducted using XLSTAT [41] for Receiver Operating Curve (ROC) analysis of variables, at a 95 per cent level of confidence.

ROC analysis plots the sensitivity and specificity of the test result against each outcome-measurement, to give an indication of a variable ability to split between cases and controls, which is a test of screening capacity for that variable and thus, its biomarker status [42] [43]. Criteria used for candidate markers to achieve biomarker status in this project were: 1) a ROC area under the curve (AUC) lower boundary of ≥0.65 at a 95% level of significance and P ≥ 0.05,with a good test having an area of 0.7 to 0.9; 2) A risk of rejection of the null hypothesis (RR-Ho), for which the ideal value is <10% (**Table S1**); 3) For this data set, a minimum Spearman' Rank Correlation coefficient of rho > 0.195, giving a P value of >0.05 and an ideal rho of >0.35 or more, giving a P value of 0.000 to <0.0001.

Though we essentially limited biomarkers identification to candidate markers that met the above criteria 1)-3), the ideal odds ratio of association with diagnosis of schizophrenia or schizoaffective disorder (**Table S1**), was an odds ratio that when divided by 3 (to compensate for a case-control study design), was either singly or in combination within a domain, able to provide an odds ratio of >2. Other ideal criteria include high percentage values for both sensitivity and specificity, (ideally > 90 percent). However in reality, there is often trade-off between sensitivity and specificity with lower values for one relative to higher values of the other.

Having identified our biomarkers using the above criteria, we then used Spearman's correlation matrix to explore and rank the relationship of the participants' symptom-ratings to their ROC-discovered biomarkers.

3.1. ROC Analysis Results

All candidate biochemical variables tested, (with the exception of MTHFR C667T polymorphism, and plasma homocysteine), produced a high area under the curve (AUC > 0.6). High serum B12 ROC AUC was marginal at

Table 5. Basic demographic data of case and control group participants (n 67).

Parameter	Number patients	Mean patients	Standard error	Number controls	Mean controls	Standard error
Age participants	67	40.5	1.3	67	45.7	1.4
Age males	37	38.6	1.4	33	46.4	1.7
Age females	30	42.9	2.4	34	45.0	2.2
Body Mass Index (BMI)	53	30.0	1.2	66	26.7	0.6
Right hand dominance %	65	92.6	2.1	67	93.1	1.7
Urine creatinine (mmol/L)	66	9.2	0.7	67	9.5	0.7
5-Hydroxyl indole acetic acid (5-HIAA)	66	4.3	0.8	67	1.6	0.1
Plasma homocysteine (umol/L)	66	10.0	0.3	66	9.5	0.3
Red cell acetylcholine esterase (U/gb Hb)	61	39.8	0.7	67	39.6	0.7
Hearing threshold (Db)	60	533.3	33.3	64	515.6	24.8
Visual threshold of near vision	61	6.3	0.5	67	5.2	0.1

0.565 and high histamine produced a poor quality ROC curve. In the field of sensory processing assessment, all candidate variables tested reached ROC identified biomarker status, with the exception of outcome measures for gap-detection and auditory-figure ground tests within the SCAN test for auditory processing disorder and outcomes for near vision using the Sussex near vision test. Catecholamine metabolites homovanillic acid (HVA) and methoxy-hydroxymandelic acid (MHMA), along with the serotonin metabolite, 5-hydroxyindoleacetic acid (HIAA), also failed to reach significance on ROC analysis.

On ROC analysis, twenty-one outcome measures achieved the required area under the curve (AUC > 0.65), risk of rejection (RR < 10%) and sufficient correlation coefficient (usually with sufficient odds ratio and/or specificity), to achieve status as a biomarker (**Table 6**, **Table S1**). These biomarkers were elevated dopamine, noradrenaline and adrenaline, along with elevated urinary hydroxyhemopyrroline-2-one (HPL/creatinine), low red cell folate, low activated B6 (pyridoxal-5'-phosphate coenzyme form), elevated ratio of percent free copper to red cell zinc and low serum vitamin D (25-OH) and elevated vitamin B12 (AUC 0.565) contributed positively to the overall nutrition-biochemical domain biomarkers. Visual processing variables were visual (symbol) span, threshold visual speed of processing performance as a percentage of age (expresses visual processing speed in terms of the visual processing system's relative age) and distance-vision on right (asymmetric binocular distance-vision acuity). Variables eligible for biomarker status within auditory processing assessment, were reverse digit span (measures verbal, auditory working memory), competing-words performance for age as a percentage of age (measures intra-cerebral dichotic processing of auditory information) and threshold speed of auditory processing as a percentage of age (expresses auditory processing speed in terms of the auditory processing systems relative to age). Six biomarkers were discovered on middle ear impedance testing. These were: threshold percentage length of the base of the stapes reflex divided by the total duration of the reflex (a measure of the strength of the stapes reflex during its maximal period of contraction), threshold stapes amplitude projected (alternative measure of stapes contraction strength), threshold time-to-off-set of the stapes reflex contraction divided by the base length (gives a measure of acoustic reflex decay), threshold ear canal volume, threshold peak middle ear pressure and threshold gradient middle ear pressure, as outlined in **Table 6**.

3.2. Symptom-Profile Analysis

In order to draw meaning from the correlation between symptoms and biomarkers, we segregated symptoms in two main ways. Firstly according to their lead (strongest) correlative biomarker and secondly according to the strength of their correlation with the three principal catecholamine components, noradrenaline, adrenaline and dopamine. In the following presentation the P values for Spearman's correlates are not presented, however significance levels correspond to the following general rules: for rho > 0.80 to 0.40, P < 0.0001, for rho 0.4 to 0.350, P = 0.000, for rho 0.350 to 0.307, P = 0.001, for rho 0.260, P = 0.007, for rho 0.255, P = 0.008, for rho 0.250, P = 0.009, for rho 0. 247, P = 0.010, for rho 0.231, P = 0.016 and for rho 0.195, P = 0.043.

Table 6. Twenty one outcome variables achieving ROC status as biomarkers for schizophrenia and schizoaffective disorder.

ROC variables	No. of Obs	AUC	ROC P value	SENS	SPEC	% RR-Ho
High dopamine (DA)	133	0.702	<0.0001	0.379	0.940	0.01
High noradrenaline (NA)	133	0.851	<0.0001	0.742	0.881	0.01
High adrenaline (AD)	133	0.844	<0.0001	0.758	0.821	0.01
High (HPL/creatinine)	133	0.696	<0.0001	0.697	0.642	0.01
High free copper to zinc ratio	133	0.611	0.022	0.470	0.746	2.19
Low B6 activation	129	0.638	0.002	0.800	0.484	0.17
High serum vitamin B12 (80% CI)	134	0.563	0.0933	0.373	0.761	18.56
Low red cell folate	133	0.654	0.001	0.591	0.716	0.10
Low vitamin D	132	0.651	0.001	0.462	0.791	0.12
Low visual span	126	0.862	<0.0001	0.831	0.821	0.01
High visual speed of processing discrepancy (VSOP) (% of age)	122	0.875	<0.0001	0.909	0.731	0.01
Poor distance vision on right	128	0.597	<0.0001	0.475	0.851	0.01
Low reverse digit span	127	0.810	0.000	0.900	0.552	0.02
High competing words discrepancy (% of pass score)	124	0.799	<0.0001	0.759	0.773	0.01
High auditory speed of processing discrepancy (ASOP) (% of age)	121	0.874	<0.0001	0.745	0.879	0.01
High threshold ear canal volume	123	0.603	<0.0001	0.367	0.825	0.01
Low threshold peak middle ear pressure	124	0.617	0.000	0.700	0.484	0.04
High threshold gradient middle ear pressure (90 per cent)	124	0.580	0.0674	0.370	0.891	6.74
High threshold stapes amplitude projected	123	0.626	0.003	0.583	0.651	0.29
Low threshold time to offset over base-length	122	0.659	0.001	0.683	0.613	0.01
High threshold percentage base-length over duration.	122	0.657	0.001	0.583	0.774	0.14

ROC: Receuver Operating Curve, AUC, No Obs, Number of observations, Area Under Receiver Operating Curve (ideally > 0.6), SENS: Sensitivity, SPEC. Specificity, ROC P value. Receiver Operating Curve variavle P value at 95% Confidence Interval, % RR-Ho. Percentage Risk Ratio for Null Hypothesis (ideally < 0.10), HPL: Urinary hydroxyhemopyrroline-2-one.

3.3. Symptom-Correlates in Relationship to Leading Biomarkers

When schizophrenia and schizoaffective disorder were segregated and ranked according to their lead (strongest) correlative biomarker, they fell into five main groups, outlined in **Tables 7-11**.

1) Low visual span **Tables 7(a)-(f)**.
2) Low visual speed of processing **Tables 8(a)-(c)**.
3) Low auditory speed of processing (ASOP) biomarker **Tables 9(a)-(d)**.
4) Elevated noradrenaline **Table 10(a)** and **Table 10(b)**.
5) Elevated adrenaline **Table 11(a)** and **Table 11(b)**.

Table 7. (a)-(f) Symptom correlates where the lead biomarker is low visual span.

(a)

Biomarker ROCs	Cognitive disorganisation rho for alpha 0.05
Low visual span (n 126)	0.632
High noradrenaline (n 133)	0.583
Low auditory speed of processing (% of age) (n 121)	0.561
Low competing words score (% of pass score) (n 124)	0.520
Low visual speed of processing (% of age) (n 122)	0.510
High adrenaline (n 133)	0.459
High dopamine (n 133)	0.405
High distance vision score on right (n 128)	0.341
Low reverse digit span (n 128)	0.335
High HPL/creatinine (n 133)	0.332
High serum B12 (n 134)	0.283
Low red cell folate (n 133)	0.258
High threshold gradient middle ear pressure (n 124)	0.248
Long threshold per cent base length/ duration (n 122)	0.242
Low vitamin D (n 132)	0.233
Low threshold time to offset/base length (n 122)	0.195
Low activated vitamin B6 (n 126)	0.180

(b)

Biomarker ROCs	Suspiciousness rho for alpha 0.05
Low visual span (n 126)	0.604
Low visual speed of processing (% of age) (n 122)	0.573
Low auditory speed of processing (% of age) (n 121)	0.568
High noradrenaline (n 133)	0.533
Low competing words score (% of pass score) (n 124)	0.477
High adrenaline (n 133)	0.458
Low reverse digit span (n 128)	0.412
High distance vision score on right (n 128)	0.390
Long threshold per cent base length/duration (n 122)	0.303
High dopamine (n 133)	0.299
Low threshold peak middle ear pressure (n 124)	0.284
Low red cell folate (n 133)	0.283
High HPL/creatinine (n 133)	0.268
Low vitamin D (n 132)	0.244
High threshold stapes amplitude projected (n 123)	0.236
Low activated vitamin B6 (n 126)	0.228
Low threshold time to offset/base length (n 122)	0.205
High Serum B12 (n 134)	0.199
High threshold gradient middle ear pressure (n 124)	0.196
High threshold ear canal volume (ECV) (n 123)	0.189
High percent free copper to zinc ratio (n 133)	0.181

(c)

Biomarker ROCs	Poor attention rho for alpha 0.05
Low visual span (n 126)	0.575
Low auditory speed of processing (% of age) (n 121)	0.551
Low visual speed of processing (% of age) (n 122)	0.546
High noradrenaline (n 133)	0.488
High adrenaline (n 133)	0.473
Low competing words score (% of pass score) (n 124)	0.455
Long threshold per cent base length/duration (n 122)	0.384
High HPL/creatinine (n 133)	0.350
Low reverse digit span (n 128)	0.336
Low red cell folate (n 133)	0.284
High threshold stapes amplitude projected (n 123)	0.266
High threshold gradient middle ear pressure (n 124)	0.261
Low threshold time to offset/base length (n 122)	0.257
High distance vision on Right (n 128)	0.247
High dopamine (n 133)	0.232
Low vitamin D (n 132)	0.227
High threshold ear canal volume (ECV) (n 123)	0.191
High Serum B12 (n 134)	0.190

(d)

Biomarker ROCs	Hostility rho for alpha 0.05
Low visual span (n 126)	0.572
High noradrenaline (n 133)	0.517
Low auditory speed of processing (% of age) (n 121)	0.504
Low visual speed of processing (% of age) (n 121)	0.419
Low competing words score (% of pass score) (n 124)	0.415
High adrenaline (n 133)	0.393
Low reverse digit span (n 128)	0.390
High dopamine (n 133)	0.321
Low vitamin D (n 132)	0.291
Low red cell folate (n 133)	0.284
High HPL/creatinine (n 133)	0.282
Low activated vitamin B6 (n 126)	0.270
Low threshold peak middle ear pressure (n 124)	0.262
High threshold gradient middle ear pressure (n 124)	0.254
High distance vision score on right (n 128)	0.241
Low threshold time to offset/base length (n 122)	0.233
Long threshold per cent base length/duration (n 122)	0.277
High threshold stapes amplitude projected (n 123)	0.195
High threshold ear canal volume (ECV) (n 123)	0.179

(e)

Biomarker ROCs	Blunted affect rho for alpha 0.05
Low visual span (n 126)	0.567
Low auditory speed of processing (% of age) (n 121)	0.548
High noradrenaline (n 133)	0.513
Low competing words score (% of pass score) (n 124)	0.489
Low visual speed of processing (% of age) (n 122)	0.482
High adrenaline (n 133)	0.441
Low reverse digit span (n 128)	0.411
Long threshold per cent base length/duration (n 122)	0.294
High distance vision score on right (n 128)	0.283
High HPL/creatinine (n 133)	0.273
High threshold gradient middle ear pressure (n 124)	0.264
Low vitamin D (n 132)	0.233
Low threshold time to offset/base length (n 122)	0.211
High threshold stapes amplitude projected (n 123)	0.205
Low red cell folate (n 133)	0.193
Low activated vitamin B6 (n 126)	0.187
High dopamine (n 133)	0.182

(f)

Biomarker ROCs	Self neglect rho for alpha 0.05
Low visual span (n 126)	0.509
High noradrenaline (n 133)	0.441
High adrenaline (n 133)	0.419
Low visual speed of processing (% of age) (n 121)	0.411
Low competing words score (% of pass score) (n 124)	0.373
Low auditory speed of processing (% of age) (n 121)	0.350
High HPL/creatinine (n 133)	0.321
Low reverse digit span (n 128)	0.311
Long threshold per cent base length/duration (n 122)	0.269
High serum B12 (n 134)	0.265
High distance vision score on right (n 128)	0.260
High dopamine (n 133)	0.250
Low vitamin D (n 132)	0.241
Low threshold time to offset/base length (n 122)	0.223
High threshold gradient middle ear pressure (n 124)	0.187
High threshold stapes amplitude projected (n 123)	0.187
Low red cell folate (n 133)	0.175

Table 8. (a) (b) Symptoms where lead biomarker is low visual speed of processing.

(a)

Biomarker ROCs	Judgment and insight impairment rho for alpha 0.05
Low visual speed of processing (% of age) (n 121)	0.598
Low visual span (n 126)	0.595
Low auditory speed of processing (% of age) (n 121)	0.587
High noradrenaline (n 133)	0.580
High adrenaline (n 133)	0.493
Low competing words score (% of pass score) (n 124)	0.465
Low reverse digit span (n 128)	0.417
High distance vision score on right (n 128)	0.397
Long threshold per cent base length/duration (n 122)	0.327
High dopamine (n 133)	0.324
High HPL/creatinine (n 133)	0.316
Low red cell folate (n 133)	0.276
Low threshold time to offset/base length (n 122)	0.226
High threshold gradient middle ear pressure (n 124)	0.223
Low vitamin D (n 132)	0.222
Low threshold peak middle ear pressure (n 124)	0.213
Low activated vitamin B6 (n 126)	0.210
High threshold ear canal volume (ECV) (n 123)	0.201
High percent free copper to zinc ratio (n 133)	0.193
High threshold stapes amplitude projected (n 123)	0.182

(b)

Biomarker ROCs	Delusions rho for alpha 0.05
Low visual speed of processing (% of age) (n 121)	0.594
Low auditory speed of processing (% of age) (n 121)	0.586
Low visual span (n 126)	0.578
Low competing words score (% of pass score) (n 124)	0.503
High noradrenaline (n 133)	0.491
High adrenaline (n 133)	0.418
High distance vision score on right (n 128)	0.410
Low reverse digit span (n 128)	0.396
High dopamine (n 133)	0.344
Long threshold per cent base length/duration (n 122)	0.325
Low red cell folate (n 133)	0.318
High HPL/creatinine (n 133)	0.258
Low vitamin D (n 132)	0.241
Low activated vitamin B6 (n 126)	0.225
High threshold stapes amplitude projected (n 123)	0.223
High serum B12 (n 134)	0.221
Low threshold peak middle ear pressure (n 124)	0.209
Low threshold time to offset/base length (n 122)	0.197

(c)

Biomarker ROCs	Anxiety rho for alpha 0.05
Low visual speed of processing (% of age) (n 121)	0.497
Low visual span (n 126)	0.491
Low auditory speed of processing (% of age) (n 121)	0.521
High noradrenaline (n 133)	0.454
High adrenaline (n 133)	0.418
Low reverse digit span (n 128)	0.324
Low competing words score (% of pass score) (n 124)	0.330
High HPL/creatinine (n 133)	0.288
Low vitamin D (n 132)	0.248
High percent free copper to zinc ratio (n 133)	0.245
High distance vision score on Right (n 128)	0.249
High dopamine (n 133)	0.234
Low red cell folate (n 133)	0.219
Low threshold peak middle ear pressure (n 124)	0.219
High threshold gradient middle ear pressure (n 124)	0.220
Long threshold per cent base length/duration (n 122)	0.192
High threshold stapes amplitude projected (n 123)	0.183
Low threshold time to offset/base length (n 122)	0.109

Table 9. (a)-(d) Symptoms where lead biomarker is low auditory speed of processing.

(a)

Biomarker ROCs	Symptom intensity rating (SIR) (severity measure) rho for alpha 0.05
Low auditory speed of processing (% of age) (n 121)	0.634
Low visual span (n 126)	0.632
Low visual speed of processing (% of age) (n 121)	0.616
High noradrenaline (n 133)	0.575
High adrenaline (n 133)	0.521
Low competing words score (% of pass score) (n 124)	0.498
Low reverse digit span (n 128)	0.446
High distance vision score on right (n 128)	0.368
Long threshold per cent base length/duration (n 122)	0.365
High HPL/creatinine (n 133)	0.342
High dopamine (n 133)	0.304
High threshold gradient middle ear pressure (n 124)	0.282
Low red cell folate (n 133)	0.278
Low vitamin D (n 132)	0.254
High threshold stapes amplitude projected (n 123)	0.242
Low threshold time to offset/base length (n 122)	0.239
High threshold Ear Canal Volume (ECV) (n 123)	0.226
High serum B12 (n 134)	0.194
Low activated vitamin B6 (n 126)	0.187

(b)

Biomarker ROCs	Unusual thought content rho for alpha 0.05
Low auditory speed of processing (% of age) (n 121)	0.581
Low visual speed of processing (% of age) (n 121)	0.580
Low visual span (n 126)	0.567
Low competing words score (% of pass score) (n 124)	0.456
High noradrenaline (n 133)	0.447
High adrenaline (n 133)	0.386
High distance vision score on right (n 128)	0.363
Low reverse digit span (n 128)	0.351
High HPL/creatinine (n 133)	0.325
Long threshold per cent base length/duration (n 122)	0.297
Low red cell folate (n 133)	0.287
High serum B12 (n 134)	0.249
Low threshold peak middle ear pressure (n 124)	0.240
High dopamine (n 133)	0.237
High threshold stapes amplitude projected (n 123)	0.223
High threshold ear canal volume (ECV) (n 123)	0.194
Low vitamin D (n 132)	0.194
High percent free copper to zinc ratio (n 133)	0.192
Low activated vitamin B6 (n 126)	0.178

(c)

Biomarker ROCs	Abstract thinking impairment rho for alpha 0.05
Low auditory speed of processing (% of age) (n 121)	0.546
Low visual speed of processing (% of age) (n 121)	0.545
Low visual span (n 126)	0.536
Low competing words score (% of pass score) (n 124)	0.505
High noradrenaline (n 133)	0.460
High adrenaline (n 133)	0.405
High HPL/creatinine (n 133)	0.395
Long threshold per cent base length/duration (n 122)	0.367
Low reverse digit span (n 128)	0.319
High dopamine (n 133)	0.272
High threshold gradient middle ear pressure (n 124)	0.267
High threshold stapes amplitude projected (n 123)	0.261
High distance vision score on right (n 128)	0.240
Low threshold time to offset/base length (n 122)	0.236
Low red cell folate (n 133)	0.230
High serum B12 (n 134)	0.219
High percent free copper to zinc ratio (n 133)	0.191
Low vitamin D (n 132)	0.186

(d)

Biomarker ROCs	Hallucinations rho for alpha 0.05
Low auditory speed of processing (% of age) (n 121)	0.545
Low visual speed of processing (% of age) (n 121)	0.502
Low visual span (n 126)	0.473
High noradrenaline (n 133)	0.454
High adrenaline (n 133)	0.397
Low competing words score (% of pass score) (n 124)	0.388
Low reverse digit span (n 128)	0.387
High distance vision score on right (n 128)	0.355
Long threshold per cent base length/duration (n 122)	0.287
Low red cell folate (n 133)	0.272
High HPL/creatinine (n 133)	0.258
High threshold stapes amplitude projected (n 123)	0.251
High threshold ear canal volume (ECV) (n 123)	0.250
Low threshold time to offset/base length (n 122)	0.230
High dopamine (n 133)	0.219
Low vitamin D (n 132)	0.200
Low activated vitamin B6 (n 126)	0.190

Table 10. (a) (b) Symptoms where lead biomarker is elevated noradrenaline.

(a)

Biomarker ROCs	Poor impulse control rho for alpha 0.05
High noradrenaline (n 133)	0.575
Low visual span (n 126)	0.515
High adrenaline (n 133)	0.485
Low competing words score (% of pass score) (n 124)	0.467
Low auditory speed of processing (% of age) (n 121)	0.451
High dopamine (n 133)	0.432
Low visual speed of processing (% of age) (n 121)	0.402
Low reverse digit span (n 128)	0.362
Low vitamin D (n 132)	0.305
High distance vision score on right (n 128)	0.304
Long threshold per cent base length/duration (n 122)	0.300
Low activated vitamin B6 (n 126)	0.278
Low red cell folate (n 133)	0.273
Low threshold time to offset/base length (n 122)	0.264
High HPL/creatinine (n 133)	0.242
High threshold stapes amplitude projected (n 123)	0.232

(b)

Biomarker ROCs	Excitement rho for alpha 0.05
High noradrenaline (n 133)	0.522
Low auditory speed of processing (% of age) (n 121)	0.493
High adrenaline (n 133)	0.437
Low visual speed of processing (% of age) (n 121)	0.437
Low visual span (n 126)	0.422
Low competing words score (% of pass score) (n 124)	0.382
High dopamine (n 133)	0.366
High distance vision score on right (n 128)	0.357
Low reverse digit span (n 128)	0.326
Low red cell folate (n 133)	0.291
Long threshold per cent base length/duration (n 122)	0.277
Low activated vitamin B6 (n 126)	0.268
Low threshold time to offset/base length (n 122)	0.223
High threshold stapes amplitude projected (n 123)	0.218
High threshold ear canal volume (ECV) (n 123)	0.206

Table 11. (a) (b) Symptoms where lead biomarker is elevated adrenaline.

(a)

Biomarker ROCs	Emotional withdrawal rho for alpha 0.05
High adrenaline (n 133)	0.503
Low visual speed of processing (% of age) (n 121)	0.470
Low visual span (n 126)	0.467
High noradrenaline (n 133)	0.432
Low competing words score (% of pass score) (n 124)	0.413
Low auditory speed of processing (% of age) (n 121)	0.407
Low reverse digit span (n 128)	0.374
Long threshold per cent base length/duration (n 122)	0.350
High threshold stapes amplitude projected (n 123)	0.317
High HPL/creatinine (n 133)	0.294
Low threshold time to offset/base length (n 122)	0.277
High threshold gradient middle ear pressure (n 124)	0.271
Low vitamin D (n 132)	0.257
High threshold ear canal volume (ECV) (n 123)	0.227
High distance vision score on right (n 128)	0.222
Low red cell folate (n 133)	0.214
Low activated vitamin B6 (n 126)	0.197
No dopamine	

(b)

Biomarker ROCs	Suicidality rho for alpha 0.05
High adrenaline (n 133)	0.341
High noradrenaline (n 133)	0.308
Low visual span (n 126)	0.264
Low auditory speed of processing (% of age) (n 121)	0.252
Low visual speed of processing (% of age) (n 121)	0.240
Low reverse digit span (n 128)	0.229
Low competing words score (% of pass score) (n 124)	0.225
Low vitamin D (n 132)	0.225
High threshold ear canal volume (ECV) (n 123)	0.222
Low red cell folate (n 133)	0.217
High dopamine (n 133)	0.198
Low activated vitamin B6 (n 126)	0.186

3.4. Symptom-Correlates in Relationship to Catecholamine Biomarkers

When symptoms were segregated according to significant positive and negative correlative strengths for the three principal ROC catecholamines noradrenaline (NA), adrenaline (AD) and dopamine (DA), symptom formation for schizophrenia and schizoaffective disorder fell into seven finely tuned graduated levels of catecholamines.

1) Symptoms with significant correlates for both elevated NA, AD and a lower correlate for DA. (Severity (SIR) (NA 0.575, AD 0.521) > DA 0.304).

2) Symptoms with significant positive correlates for NA and AD, but low DA correlates.

3) Symptoms with elevated NA (n 133, rho 0.5, $P < 0.0001$), compared with weaker AD and DA.

4) Symptoms with strongest correlation for adrenaline (AD) in a setting of a weak dopamine correlate.

5) Decreasing correlative strengths for dopamine (DA) n 133, (together with strong NA and AD correlates) and symptoms demonstrating no significant correlates for the dopamine biomarker.

6) Symptoms demonstrating an absence of significant correlates for the dopamine biomarker.

7) Symptoms with significant positive correlates for elevated dopamine biomarker.

8) Symptoms with generalised low or absent correlates for all catecholamines.

These graduated levels of catecholamines are presented in relationship to their biomarker correlates in **Table 12**.

3.5. Symptom-Correlates for the Oxidative Stress Marker, Elevated Haeme-Hydroxy-Pyrroline-2-One (HPL)

Elevated HPL/creatinine levels correlated with high DA ROC (n 131, rho 0.270, P 0.002) and high HIAA ROC (AUC 0.677 at 70% level of significance, n 131, rho 0.200, P 0.022) and high NA ROC (n 131, rho 0.232, P 0.008) and high AD ROC (n 131, rho 0.241, P 0.006). Significant positive correlates were obtained for HPL with clinical severity (SIR index) and a number of negative symptoms: Abstract thinking impairment 0.395, poor attention 0.350, clinical severity (SIR) rho 0.342, cognitive disorganisation 0.332, unusual thought content 0.325, disturbed volition 0.327, lack of spontaneous conversation 0.325, self-neglect 0.321, judgment and insight impairment 0.316, social avoidance 0.314, poor rapport 0.305, emotional withdrawal 0.294. Most notably, HPL held a significant correlation with the symptom of anxiety (n 133, rho 0.245, P 0.004) and marginally-significant correlations with reverse digit span (working memory) deficit (n 127, rho 0.204, P 0.022) and competing words (dichotic listening) deficit (n 123, rho 0.183, P 0.043). Additional marginal correlates were found for high free copper:zinc ratio (n 129, rho 0.171, P 0.053).

3.6. Symptom-Correlates for Peripheral Sensory Dysfunction

Impaired long distance vision (on right) was significantly related to all symptoms with the exception of negative

Table 12. Symptoms segregated according to catecholamine biomarker levels.

Catecholamine ROC variables	Symptom correlates (rho) at 95% confidence level.
1) Symptoms with significant correlates for both elevated NA, AD and DA, but DA correlates were lower.	Severity (SIR) (NA 0.575, AD 0.521) > DA 0.304 Delusions (n 133, NA 0.491, AD 0.418) > DA 0.344
2) Symptoms with significant positive correlates for NA and AD, but low DA correlates.	Poor attention (NA 0.488, AD 0.473) > DA 0.232 Abstract thinking impairment (NA 0.460, AD 0.405) > DA 0.272 Unusual thought (NA 0.447, AD 0.386) > DA 0.237 Self-neglect (NA 0.441, AD 0.419) > DA 0.250 Anxiety (NA 0.454, AD 0.418) > DA 0.234 Suicidality (AD 0.341, NA 0.308) > DA 0.198
3) Elevated NA n 133, rho 0.5, P, 0.0001 compared with weaker AD and DA	Cognitive disorganisation NA 0.583 > (AD 0.495 > DA 0.405) Judgment and Insight impairment NA 0.580 > (NA 0.493 > DA 0.324) SIR 0.575 Poor impulse control NA 0.575 > (AD 0.485 > DA 0.432) Excitement NA 0.552 > (AD 0.437 > DA 0.366) Suspiciousness NA 0.533 > (AD 0.458 > DA 0.299*) Hostility NA 0.517 > (AD 0.373 > DA 0.321) Blunted affect NA 0.513 > (AD 0.441> DA 0.182*) Hallucinations NA 0.454 > (AD 0.397 > DA 0.219*)
4) Strongest correlative strength is for adrenaline (AD) in a setting of weak dopamine correlate.	SIR 0.521 Emotional withdrawal AD 0.503 > NA 0.432 > DA 0.00* Suicidality AD 0.341, NA 0.308) > DA 0.198*
5) Decreasing correlative strengths for dopamine (DA) n 133, (in a setting of strong NA and AD correlates demonstrating gradation from excitement to suicidality and emotional withdrawal.	Motor hyperactivity 0.451 Poor impulse control 0.432 Cognitive disorganisation 0.405 Excitement 0.366 Bizarre behaviour 0.363 Grandiosity 0.361 Elated mood 0.385 Hostility 0.321 Self-neglect 0.250 Unusual thought content 0.237 Anxiety 0.234 Judgment and Insight impairment 0.234 Poor attention 0.232 Suspiciousness 0.229 Hallucinations 0.219 Suicidality 0.198 Emotional withdrawal 0.00
6) Symptoms demonstrating an absence of significant correlates for the dopamine biomarker.	Emotional withdrawal, blunted affect, depressed mood, disturbed volition, motor retardation, passivity/apathy, symptom severity (SIR), lack of spontaneous conversation, stereotypic thinking, mannerisms and posturing, guilt, dissociative symptoms (experiencing blank periods, unreal feelings, being outside of oneself and other identities within self), ideas of reference and control, distractibility, thought pre-occupation, lack of spontaneous conversation, poor rapport, social avoidance and a history of abuse.
7) Symptoms with significant positive correlates for elevated dopamine biomarker.	Motor hyperactivity 0.345. Elevated mood 0.258. Grandiosity 0.243. Motor hyperactivity 0.345.
8) Symptoms with generalised low or absent correlates for *all* catecholamines.	Experiencing blank periods, (usually considered a dissociative symptom).

symptoms of depressed mood, disturbed volition, suicide and dissociative symptoms. In contrast, low, significant correlates were largely found for positive symptoms with delusions 0.410, thought preoccupation 0.400, judgment and insight impairment 0.397, suspiciousness 0.390, motor-hyperactivity 0.371, symptom intensity rating (SIR) 0.368, grandiosity 0.368, unusual thought 0.363, excitement 0.357, hallucinations 0.355, cognitive disorganisation 0.341, judgment and insight impairment 0.313, bizzare behaviour 0.311 and poor impulse control 0.304.

Middle ear dysfunction biomarkers demonstrated correlates with schizophrenia and schizoaffective disorder-that included symptoms of disturbed communication:

1) Low attainment of threshold peak middle ear pressure (sensitivity 70%, SIR n 124, rho 0.184, P 0.04), correlated with symptoms of suspiciousness 0.284, hostility 0.262, unusual thought content 0.240, delusions 0.221, thought pre-occupation 0.219, judgment and insight impairment 0.213. This variable also correlated significantly with pre-aged, delayed auditory speed of processing (n 117, rho 0.243, P 0.008) and with elevated noradrenaline levels (n 123, rho 0.220, P 0.014).

2) High external canal volume (ECV) held a biomarker specificity of 82% and for 123 observations, correlated with hallucinations 0.250, emotional withdrawal 0.227, SIR 0.226, depressed mood 0.225, social avoidance 0.244, suicidality 0.222. This variable also correlated specifically with delayed auditory speed of processing (n 116, rho 0.284, P 0.02).

3) High threshold gradient middle ear pressure, may be compensative to 1. This biomarker held a ROC specificity of 89% and was significantly correlated with SIR (n 124, 0.282, P 0.005) and with symptoms of poor rapport 0.411, lack of spontaneous conversation 0.282, suspiciousness 0.284, hostility 0.262 and unusual thought 0.240.

4) There was a tendency for increased strength and duration of stapes sound-dampening, acoustic reflex (possibly compensative to 1 and 2), to be also related to negative symptoms:

a) Long % base-length compared with total duration of the maximal contraction of the stapes muscle at threshold is indicative of a strong, long-duration stapes contraction. This biomarker held a sensitivity of 77% for 122 observations and correlated positively with poor attention 0.384, delusions 0.325, suspiciousness 0.303, poor impulse control 0.342, anxiety 0.341, abstract thinking impairment 0.367, SIR 0.365, distractibility 0.331, lack of spontaneous conversation 0.339, stereotypic thinking 0.336, judgment and insight impairment 0.327, poor impulse control 0.303, disturbed volition 0.301, social avoidance 0.301. This variable representing increased duration of stapes contraction also significantly correlated with elevated levels of both noradrenaline (n 121, rho 0.212, P 0.020) and adrenaline (n 121, rho 0.264, P 0.003), respectively.

b) High stapes projected amplitude at threshold contraction response relates to increased strength of stapes contraction as it dampens sound by landing on the oval window of the cochlea. This biomarker was also significantly correlated with SIR (n 123, rho 0.242, P 0.009, and with symptoms of emotional withdrawal (n 123, rho 0.317, P 0.001) and stereotypic thinking (n 123, rho 0.290, P 0.009).

c) Low threshold time to offset/base length (n 122) indicates a protracted stapes contraction, that also correlated significantly emotional withdrawal 0.277 and with similar symptoms to a), such as distractibility 0.268, poor impulse control 0.264, poor rapport 0.259, poor attention 0.257, disturbed volition 0.251, anxiety 0.243, social avoidance 0.241, abstract thinking impairment 0.236 and SIR 0.239. This variable also came close to significant correlation with delayed-for-age auditory processing speed (n 115, rho 0.161, P 0.071).

3.7. Symptom-Correlates for Vitamin and Mineral Co-Factor Dysfunction

Nutritional-Biochemical variables significantly correlated with neurotransmitter ROC variables for high dopamine (n 124, rho 0.301, P 0.001), high noradrenaline (n 124, rho 0.381, $P < 0.0001$ and high adrenaline (n 124 rho 0.302, $P < 0.0001$). In this setting, low, significant positive correlations for symptoms in relationship to biochemical and nutritional biomarkers were found at 95% confidence level. These included:

1) Elevated vitamin B12 ROC n 134, which correlated with symptoms of cognitive disorganisation 0.283, self-neglect 0.265, abstract thinking impairment 0.219, bizarre behaviour 0.251, unusual thought content 0.249, but had a low correlate with severity (SIR 0.194).

2) Low vitamin D ROC (n 132), which correlated with symptoms of poor rapport 0.349, uncooperativeness 0.315, poor impulse control 0.305, social avoidance 0.300, hostility 0.291 and SIR 0.254.

3) Low red cell folate (n 133), which correlated with symptoms of delusions 0.318, excitement 0.291, uncooperativeness 0.290, SIR 0.278.

4) Low activated B6 (n 126), which correlated with poor impulse control 0.278, hostility 0.270, excitement 0. 268, suspiciousness 0.228, uncooperativeness 0. 242, grandiosity 0.206 and SIR 0.187.

5) High percent free copper: zinc ratio biomarker (n 133), which marginally correlated with symptoms of suspiciousness (rho 0.181, P 0.038), abstract thinking impairment (rho 0.191, P 0.028), anxiety (rho 0.228, P 0.008), unusual thought (rho 0.192, P 0.027), judgment and insight impairment (rho 0.193, P 0.026), thought preoccupation (rho 0.188, P 0.030), distractibility (rho 0.192, P 0.027) and SIR (rho 0.179, P 0.040).

4. Discussion

This research brings together ROC-defined biomarkers for schizophrenia and schizoaffective psychosis and investigates their correlation profiles in relation to discrete symptoms of these conditions.

The study indicates that finely-tuned relative-strengths between noradrenaline, adrenaline and dopamine (NA, AD and DA) levels are critical in differentiating symptom-formation in schizophrenia and schizoaffective disorder. The graduated difference between the elevated NA biomarker and the level of DA appears to turn symptom correlates from manic (with high DA) to disorganized and distracted (high NA and somewhat lower AD and DA), to depressive and negative (low DA ± high NA, or low DA alone), to dissociative symptoms such as experiencing "blank periods" that are characterised by generally low or absent catecholamine correlates. DA gives high frequency conditioning to glutaminergic synapses in order to potentiate goal-directed sensory information flow around the cortex, whilst high NA raises arousal and vigilance and AD promotes flight [44]. In moderate amounts NA also promotes sensory attention, however disproportionately excessive amounts of NA are reported to suppresses and disrupt DA-facilitated information flow around the cortex [45]-[53]. In this project the elevated NA biomarker correlated strongly with symptom severity as measured by the Symptom Intensity Rating (SIR) index. Elevated NA also correlated significantly with symptoms representing sensory flow disruption and symptoms indicative of sensory-disconnectivity within the cortex, such as impaired attention and cognitive disorganisation. We believe that noradrenergic disrupted internal sensory processing strain then activates the hypothalamic pituitary adrenal axis (HPA) [54]-[56], stimulating further catecholamine synthesis and setting in motion a vicious cycle of raised catecholamine output related to symptom formation in antipsychotic-free and antipsychotic treated persons, alike [57]. In this setting of increased catecholamine requirement, any deficiency of vitamin B6 due to poor nutrition [58] will be exacerbated by its increased requirement as a cofactor by the DA-synthesis-enzyme tyrosine hydroxylase and also for NA-synthesis by the enzyme dopa-decarboxylase [59] [60], (**Figure 1**). Vitamin B6 is also necessary to combat oxidative stress by the brain anti-oxidant glutathione, which utilises B6 in its synthesis via cystathione beta synthase (CBS) and cystathionase [61], (**Figure 1**). Low folate precursor can theoretically reduce synthesis of upstream S-adenosylmethionine (SAMe) [62] and if SAMe is depleted, then the catechol-o-methyl transferase (COMT) enzyme is inhibited, since SAMe acts as a necessary cofactor for this enzyme which resides at the end-stage of catecholamine metabolism [63]. Therefore, in the absence of its cofactor, catecholamines will be elevated. The predominance of noradrenaline over dopamine and adrenaline in this study may then be further explained by the role of free copper in promoting the synthesis of noradrenaline from dopamine (**Figure 1**). In this setting, low folate (which serves as a remote precursor of SAMe), may also contribute to SAMe unavailability and by lessening conversion of adrenaline from noradrenaline, further contribute to noradrenaline excess (**Figure 1**, **Table 12**). Taken together, these two dynamics explain why SAMe unavailability allows prominence of noradrenaline (NA) in this project's findings, with relatively lesser levels of dopamine (DA) and adrenaline (AD) in the presence of low folate and high free copper. These are interesting integrated findings which may provide an alternative explanation for the role of the COMT enzyme which has been already implicated in schizophrenia [64] [65].

Elevated noradrenaline's ability to disrupt DA's promotion of the sensory signal around the cortex from frontal to temporal-hippocampal areas, fits well with the findings of this study where high NA correlates with symptoms of poor attention, disorganisation, self-neglect and delusions, provide residual evidence of front-hippocampal disconnectivity. At the same time noradrenergic fronto-parietal sensory signal disruption [45]-[53] isolates the frontal cortex and also the default network [66] [67], resulting in poor frontal surveillance symptoms such judgement impairment, mixed with hostility, symptomatic of frontally unrestrained amygdala function. Our findings have also demonstrated that such disconnectivity may also be induced by oxidative stress (with elevated HPL), with vitamin deficits and elevated free copper also known to cause neuronal damage in sensory-processing circuits [68] [69].

Urinary HPL is thought to arise under conditions of extreme oxidative stress where it is excreted as a by-product of haeme-related porphyrin synthesis [70]-[74]. In this study, HPL established its value as a biomarker that is notably linked with clinical severity, with elevated neurotransmitter levels, with impaired auditory working memory, with dichotic deficit, with delayed auditory processing systems and also with significant negative symptoms and anxiety. Taken together, this gives the impression that oxidative stress aligns with and potentiates the action of elevated catecholamines in disrupting sensory processing circuits in the cortex, leading to anxiety and rebound HPA activation, with potential for a vicious cycle of elevated catecholamine synthesis. In literature, elevated HPL is reported to relate to vitamin B6 and zinc deficiency, since HPL is a breakdown product from

porphyrin synthesis and such synthesis is impaired in a setting of vitamin B6 and zinc unavailability [75]. Despite such reports, our study only found marginal correlates for elevated HPL with high free copper: zinc ratio (**Figure 2**).

In keeping with their various influences on catecholamine biochemistry (**Figure 1**), biomarkers for nutritional deficits such as vitamin deficiency (D, B6, folate) and low zinc, correlated significantly with elevated catecholamine levels and appear to impact a diffuse range of predominantly positive symptoms. Folate has been reported to be low in schizophrenia [76] and low zinc has been found related to noradrenaline excess [77]. Low vitamin D is an established finding in schizophrenia [78] and in this study, its biomarker correlated highly with auditory processing biomarkers and moderately with middle ear and elevated catecholamine biomarkers. A proposed mechanism linking elevated catecholamines and low vitamin D levels occurs via parathyroid hormone, the secretion of which is primed by catecholamines, which function to encourage parathyroid hormone action with release of calcium (Ca^{2+}) ions from neuronal cells-an effect that relates to neuronal excitotoxicity, but which is directly opposed by the action of vitamin D [79]-[81]. Thus catecholamine-induced draw-down on body reserves of this over-utilised vitamin together with the indoor lifestyle and lack of sun exposure that may accompany the schizophrenia condition, may work together to potentiate these effects.

Evidence of significant correlative relationships between elevated catecholamines and auditory and visual processing speed and working memory deficits are postulated to occur via a vicious cycle of catecholamine excess, whereby NA is progressively elevated until fronto-temporal disconnectivity occurs [82]. Auditory and visual working memory deficits (low reverse digit span and visual span), dichotic listening deficits and pre-aged visual and auditory processing speed deficits not attributable to anti-psychotic medication may occur in this manner and have been reported in schizophrenia [83]-[91]. In this study, we have established that these deficits

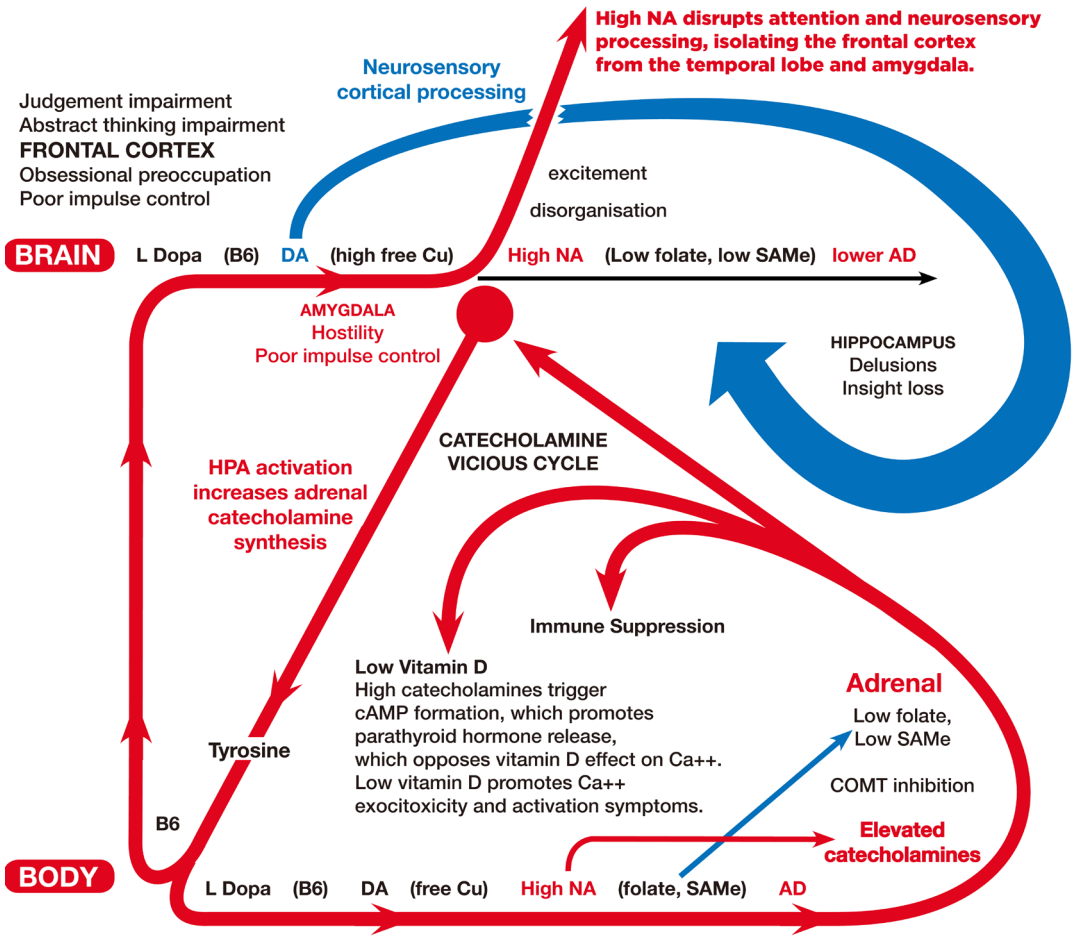

Figure 2. Vicious cycle of NA and catecholamines, in relationship to vitamin and mineral cofactor biomarkers.

qualify as biomarkers, which inter-correlate with each other, with elevated catecholamines, with delay in both auditory and visual speed of processing and also with clinical symptom severity within the research patient population.

In keeping with this noradrenergic fronto-temporal disconnection hypothesis, low visual span may represent ramped-up noradrenaline-induced disconnectivity, reducing the action of the higher visual cortical magnocellular pathway and forcing reliance on the lower parvocellular visual pathway within the default brain network [92]. In this study, the range of symptoms correlating with reduced visual span, appear to reflect absence of the magnocellular pathway since this symptom repeatedly combines with symptoms of parietal inattention, disorganization and delayed visual speed of processing. At the same time, hippocampal isolation arising from hippocampal disconnection from frontal rationalization processes, allows internal semantic confabulation, resulting in delusional thought-formation. At the sub-cortical level the amygdala is similarly isolated from frontal cortical inhibition, with resultant hostility.

Middle ear ROC reflex findings of elevated tympanic contraction gradient and increased volume of the external auditory canal—yet collateral attainment of low middle ear peak pressure—indicate that middle ear patency problems associated with tympanic membrane pathology or Eustachian tube dysfunction are an integral part of schizophrenia and schizoaffective disorder [39]. Significant correlations for some of these variables with elevated catecholamine biomarkers may further explain catecholamine-related acoustic-hypersensitivity findings reported by Adler *et al*. in 1990 [93]. Our findings imply that in the setting of elevated catecholamines, tympanic muscle contraction facilitates hearing by undergoing compensatory over-contraction, which is then transmitted across the middle ear to influence stronger, delayed stapes muscle contraction at the other end of the ossicle chain. Such stronger stapes contraction then over-dampens and delays the passage of sound as it enters the cochlear. This may be the peripheral cause of the postulated pre-attentive auditory processing deficit in schizophrenia [94] [95]. Our findings also demonstrated significant ROC correlations between high external ear canal volume, low attainment of middle ear pressure and delayed offset of stapes contraction in relationship to delayed auditory speed of processing, confirming our understanding that the overall outcome of exacerbated tympanic muscle contraction transmission to the stapes muscle is increased delay of the auditory signal as it enters the cochlear and that this significantly relates to delayed auditory speed of processing. It is therefore of further interest that our study found prominent correlation of these middle ear auditory dampening and delay biomarkers with elevated NA and AD and negative symptoms of emotional withdrawal and lack of verbal spontaneity. In contrast, other peripheral biomarkers, such as long-distance vision abnormality correlate with clinical symptom severity and activated symptoms of motor-hyperactivity, grandiosity, excitement, bizzare-behaviour and hallucinations. If the brain does not get signals from its senses it constructs them internally. These peripheral findings represent unmet needs within the patient participants in our study and, imply that broader clinical assessment and targeted remediation of these conditions may assist symptom reduction in schizophrenia and schizoaffective disorder.

5. Limitations

In order to exclude confounding factors and isolate the functional core of schizophrenia, many exclusion criteria were applied to sample selection. This reduced available participants resulting in a small discovery data-set. Small sample size together with non-normal data distribution for many variables precluded use of principal component, multivariate and cluster analysis.

Though case-control studies are suitable for discovery projects for low prevalence disorders such as schizophrenia, their cross-sectional design has inherent susceptibility to prevalence and selection bias [96] [97]. Though differences in selection processes in this study were also inherent to the design, there was occasional cross-over in recruitment methodology and recruitment bias may have been offset by the random nature of recruitment success, as there was a refusal to consent to consent ratio of 4 to 1, for approached patients and controls alike.

Although most ROC variables identified in our study achieved a robust odds ratio of much over 2, some statisticians claim that odds of association results may be inflated by up to three times with a case-control design. [98]. For this reason all biomarker odds ratios were submitted to a “division by three” test, as described in the Data analysis and results Section 3, page 86.

The application of this design to only one bracket of psychotic disorders (schizophrenia and schizoaffective disorder) in this project, limits understanding of disease specificity across other mental illness states. A large

cohort study with fully-blinded investigators or a prospective, multi-site-clinic trial on a single series consecutive patients with an emphasis on collecting symptomatic data from ultra-high risk medication naïve subjects, will therefore be required to validate these findings.

Though elevated urinary monoamine biomarkers identified in this study related to psychiatric symptoms in understandable ways, fully-synthesized monoamines do not cross the blood-brain barrier even though their precursor substance, L-Dopa, is capable of this transition [99]. Though spot-urine collection for peripheral monoamine analysis achieved good face validity with respect to symptom correlates in this study, this method of urine collection has attracted criticism [100]. The spot urine collection method is however practical in a psychiatric and pediatric settings [101] and is gaining ground as a useful analytic method relating to body biochemistry [102]. Although the oxidative-stress related molecule urinary HPL earned its place as a biomarker in this study, an understanding of this molecule's synthesis is incomplete and additional biomarkers for oxidative stress would assist to quantifying the role of oxidative stress in schizophrenia and schizoaffective disorder.

Although rigorous efforts were made to exclude substance-related diagnoses, it is known that alcohol misuse is often under-reported by patients [103], and has a known association with low folate, high B12, elevated catecholamine levels and reduced methylation [104] [105]. It was not possible to control for participant smoking in this project and smoking also has a reported effect on monoamine oxidase levels that can theoretically influence monoamine levels [106]. Though fasting biological samples were collected for this study, there was no longer term control or assessment of dietary intake, which in principle, could affect reserves of vitamins, minerals and monoamines [107].

This research occurred with antipsychotic medicated patients who met strict criteria for such medication.

Nevertheless, the effect of medication on catecholamine results cannot be fully excluded as a confounding factor. In support of the fact that medications have not influenced these catecholamine results is the fact that there is widespread research evidence that antipsychotic medications increase catecholamine metabolic turnover and reduce dopamine and noradrenaline levels [108]-[110]. Also, the elevated urinary catecholamine biomarkers identified in this study strongly correlated with Symptom Intensity Rating (SIR), as a measure of clinical severity and they also demonstrated face validity by relating to the different psychiatric symptoms in a clinically meaningful manner. The fact that elevated catecholamines reached biomarkers status in this project and demonstrated their relationship to symptom-severity and sensory disconnectivity, means that many current antipsychotic medications may be failing to counteract high levels of catecholamines and reduce the impact of sensory disconnection on psychosis. In this context of potential for residual symptom-formation and treatment-resistance, it is therefore understandable that there is increasing pharmacological interest in noradrenergic targets for treatment of cognitive deficits and other aspects of schizophrenia condition [111].

6. Conclusions

Though it is expected that hundreds of biomarkers for schizophrenia will eventually be discovered, this project provides an advanced understanding of psychosis by correlating the mood, perceptual and behavioural symptoms of schizophrenia and schizoaffective disorder with twenty-one quantified biomarkers.

Examining symptoms in relationship to biomarkers reveals that schizophrenic and schizoaffective disorder symptoms consist of a conglomerate of biochemical and neurophysiological dysfunctions related to dysregulation of visual and auditory sensory processing and neurochemistry relating to the biosynthesis and metabolism of catecholamines. Through the remote and direct action of cofactors associated with catecholamine synthesis and metabolism, noradrenaline levels are generally elevated which delays and disrupts cortical visual and auditory sensory processing pathways. This in turn activates the hypothalamic pituitary adrenal axis to produce further catecholamines. In this process, elevated free copper facilitates noradrenaline synthesis at the expense of dopamine and also promotes oxidative stress, whilst vitamin B6 over-utilisation reflects HPA pressure for dopamine synthesis and such lack also accentuates oxidative stress. Low folate remotely influences the ability of catecholamines to be metabolized by COMT via its cofactor, SAMe. Under the variable influence of these cofactors, altering ratios between noradrenaline, adrenaline and dopamine correlate meaningfully with mood, attention and behavioural effects of these catecholamines. In this setting, a predominance of very high-noradrenaline causes fronto-temporal disconnectivity resulting in symptoms of delayed, disrupted and disorganised visual and auditory attention, mixed with frontal disconnection and disinhibition symptoms of poor judgement, poor executive function, poor working memory and poor impulse control. Isolated temporal regions are then left to function alone with impaired insight and delusions, whilst the isolated amygdala within the unmasked default

network manifests as symptoms of agitation, aggression and hostility.

This research provides a new understanding of the substructure of many symptoms within the context of psychosis. In particular, biomarkers correlate and combine to underlie symptoms and behaviours of key relevance within the clinical setting, such as suicidality, hostility and auditory hallucinations.

In this context, suicidality is seen to relate to high adrenaline levels representing the flight impulse, together with low-dopamine-related symptom of emotional withdrawal. In addition high noradrenaline levels convey the capacity to act-out on the basis of these impulses and symptoms, in the setting of a psychotic break in sensory processing with dichotic listening deficit, visual and auditory processing deficits, middle ear pathology and deficiency of all three nutrients—D, B6 and folate.

In the context of predicting and preventing aggression in psychosis, the biomarker profile of hostile, aggressive behaviour is of particular interest. Hostility is seen to relate to high noradrenaline levels and has a strong correlate with the symptom of blunted affect. Hostility also relates to impaired visual span and auditory processing difficulty and has underlying pathology relating to vitamin D deficiency and middle ear pathology. Such findings may be helpful for prediction of hostility in a clinical or forensic context.

Auditory hallucinations are seen to relate to high levels of both noradrenaline and adrenaline with delays in both auditory and visual processing speed, together with reduced visual span. In addition, dichotic listening deficit, middle ear pathology, low folate, low vitamin D and B6 contribute to the constellation of disorders and dysfunctions that subtly accumulate to produce this distressing symptom.

Anxiety is also seen to relate to elevated noradrenaline and adrenaline levels, with impairment of visual span and visual processing speeds to a greater extent than delayed auditory processing. In addition, auditory working memory and dichotic listening deficits impair sensory processing. Underlying this dysfunction resides peripheral abnormalities of long distance vision and the middle ear function, in addition to oxidative stress and low vitamin D and folate.

Biomarkers of acoustic reflexes relating to middle ear pathology, distance vision deficits, dichotic listening disorder and biochemical-nutritional deficits generally represent unmet needs within the clinical population assessed. This implies that there is scope for broader clinical assessment and remediation of persons with schizophrenia and schizoaffective disorder.

Biomarkers that correlate with discrete symptoms or sets of symptoms have therapeutic potential for targeted correction towards cure. They inform about the structure of psychosis, symptom-formation and the patterns in which symptoms present, allowing clinicians greater confidence in diagnosis and management of discrete, resistant, difficult or dangerous symptoms. Such multi-domain understandings provide a template for new biological system of symptom-interpretation in serious mental illness states. In the wider social context, the demonstration of clear biological underpinnings for schizophrenia and schizoaffective psychosis will reduce social stigma associated with these conditions and improve outcome expectations for patients and their families.

Acknowledgements

The authors acknowledge the additional support of Ms. Natasha J. Radcliffe (MHumNutr, GradDipSocSci, GradCertDisStud, BA (Sociology)) who undertook control stratification and enrolment with analysis of variable distributions, proxy standard errors, ROC analysis, odds ratio analysis and Spearman's correlation analysis. Statistician Graeme Tucker (B.Sc.) also conducted final review statistical analysis. Queen Elizabeth Hospital academic and secretarial staff, nursing staff, Registrars and Consultants. Dr Geoffrey Schrader MBBS, M Clin Sc, PhD, FRANZCP and Dr Helen Tingay FRANZCP. Western Mental Health Service, Janet Grant (Population Research and Outcome Studies (PROS) and North West Adelaide Health Study assistance with participant selection. Laboratory support from Dr. Michael Metz BS, MD, FAAP, MAACB, FRCPA at Clinpath Laboratories and Dianne Zercher (TQEH Biochem Laboratory) and Dr Malcolm Whiting at SA Pathology. General support from Professor Robert Adams Head of Department of Medicine TQEH, Professor Richard Ruffin AM, Judith Snowden, Helen Goldsack, Dr Marilyn Dyson MBBS, MHP, DRCOG, Dr Sinclair Bode MB BS FACNEM, Veronica Steer B AppSc (OT), Jan Pollard (technical audiology support), Dr N. Williams (Ophthalmologist FRANZCO). Graphic support Adlab SA.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships

that could be construed as a potential conflict of interest.

A provisional patent application was filed by the author at the conclusion of data-collection and initial analysis, in December 2013.

Authors Contributions

As Chief Researcher, Dr Stephanie Fryar-Williams MB BS. BSc (Biochem/Pharmacol) FRANZCP is an Honorary Research Fellow of the University of Adelaide. Dr Fryar-Williams conceived the project, selected the candidate markers and designed the research protocols, oriented staff and raters in ward and clinic settings, managed and supervised the recruitment of case and control participants, prepared the data-set for analysis by data-transformation, supervised ROC and other statistical analysis, directed and interpreted data analysis within biochemical, neurological and psychiatric theory and wrote the paper.

As a previous Clinical Director of Psychiatry at the Queen Elizabeth Hospital, Woodville SA., Dr Jorg Strobel MD (Bonn). Spec Psych, Psychotherapy FRANZCP facilitated the laboratory, ward and community organisation and coordination components of the project and revised past and final papers for publication.

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Supplementary Tables

Table S1. ROC results for schizophrenia and schizoaffective disorder, with odds ratios.

ROC variables and domains	No. Obs	AUC	Odds ratio	Odds ratio P	ROC variables and domains	No. Obs	AUC	Odds ratio	Odds ratio P
Low visual span	126	0.862	22.46	<0.0001	High dopamine	133	0.702	9.60	<0.0001
High visual speed of processing iscrepancy (% of age)	122	0.875	27.22	<0.0001	High noradrenaline	133	0.851	21.25	< 0.0001
Poor distance vision on right	128	0.597	5.17	0.0001	High adrenaline	133	0.844	14.32	< 0.0001
Visual domain	**120**	**0.915**	**41.48**	**<0.0001**	**Catecholamine domain**	**133**	**0.859**	**4.12**	**<0.0001**
Reverse digit span	126	0.862	11.00	<0.0001					
High competing words discrepancy (% of pass score)	124	0.875	10.69	<0.0001	Low vitamin B6 activated	129	0.638	3.75	0.0009
High auditory speed of processing discrepancy (% for age)	121	0.874	21.23	<0.0001	High free copper to zinc ratio	133	0.611	2.60	0.0104
Auditory domain	**119**	**0.891**	**29.57**	**<0.0001**	Low red cell folate	133	0.654	3.64	0.0005
					Low vitamin D	132	0.651	3.24	0.0026
High (HPL/creatinine) model	133	0.696	4.12	<0.0001	High serum B12 (80% CI)	134	0.565	1.89	0.0933
Oxidative stress domain	**133**	**0.696**	**4.12**	**<0.0001**	**Nutrition-biochemistry**	**126**	**0.797**	**8.5**	**< 0.0001**
High threshold ear canal volume	123	0.603	2.74	0.0181					
Low threshold peak middle ear pressure	124	0.617	2.19	0.0369					
High threshold gradient middle ear tympanic contraction (90 per cent CI)	124	0.580	3.77	0.0064					
High threshold stapes amplitude projected	123	0.626	2.61	0.0099					
Low threshold time to offset over base-length	122	0.659	3.42	0.0013					
High threshold percentage base-length over duration	122	0.657	4.80	0.0001					
Middle ear domain	**120**	**0.738**	**4.28**	**0.0001**					

Table S2. Distribution summary for biomarker variables.

ROC variable	No. Obs	Minimum	Maximum	Mean	SD	Median	MAD (mean absolute deviation)	Fitted distribution
Visual domain								
Low visual span	134	0.00	8.00	5.476	1.35	6.00	1.00	Pert
High visual speed of processing discrepancy (% of age)	134	−90.00	207.69	6.01	54.44	−5.55	30.24	Log normal
Poor distance vision on right	134	0.00	36.00	7.98	6.03	6.00	1.50	Chi-square

Continued

Auditory domain								
Reverse digit span	134	2.00	8.00	4.22	1.33	4.00	1.00	Gamma (3P)
High competing words discrepancy (% of pass score)	134	−69.23	50.00	0.55	22.61	3.84	15.38	Johnson SB
High auditory speed of processing discrepancy (% of age)	134	−100.00	220.00	−3.83	56.68	−18.00	31.12	Gen. extreme value
Catecholamine domain								
Dopamine	134	45.00	358.00	142.4	53.64	129.0	32.00	Log-logistic
Noradrenaline	134	3.00	106.00	25.27	18.53	19.00	9.00	Johnson SB
Adrenaline	134	0.00	27.00	4.413	5.10	2.00	1.00	Log-logistic
HPL/creatinine	134	0.35	40.04	4.586	5.94	2.47	1.18	Burr
Nutrition-biochemistry domain								
Free copper to zinc ratio	134	−1.85	1.60	0.267	0.52	0.31	0.31	Log-logistic
B6 activation	134	12.80	1570.0	140.4	164.6	90.00	25.00	Gen. extreme value
Red cell folate	134	506.00	3291.0	1788.	448.7	1733.	236.00	Log-logistic
Serum B12	134	42.00	1388.0	406.1	178.5	367.0	104.00	Gen. extreme value
Vitamin D	134	13.00	149.00	52.46	22.15	52.00	14.00	Dagum
Middle ear domain								
Threshold ear canal volume	134	0.000	8000	1.213	0.722	1,100	0.3000	Gen. extreme value
Threshold peak middle ear pressure	134	−275,000	20,000	−29.07	44.48	−15.00	10.0000	Cauchy
Threshold gradient middle ear pressure (90 percent)	134	0.000	165,00	60.79	31.37	55.00	20.0000	Log-logistic
Threshold stapes amplitude projected	134	0.000	30.000	11.81	6.662	1.000	4.000	Gumbel max
Threshold time to offset over base length	134	0.000	80.000	13.03	22.04	1.760	0.6300	Burr
Threshold percentage base length over duration	134	0.000	77.770	28.58	23.75	29.28	20.7150	Gen pareto

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