Schizophrenia Research 213 (2019) 87–95

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Transcriptional changes in the stress pathway are related to symptoms in schizophrenia and to mood in schizoaffective disorder

Cynthia H. Lee [a, b], Duncan Sinclair [c], Maryanne O'Donnell [a, d], Cherrie Galletly [e, f, g], Dennis Liu [e, f], Cynthia Shannon Weickert [a, b, h, 1], Thomas W. Weickert [a, b, h, *, 1]

[a] School of Psychiatry, Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia
[b] Schizophrenia Research Laboratory, Neuroscience Research Australia, Randwick, NSW 2031, Australia
[c] University of Tasmania, Hobart, Australia
[d] Prince of Wales Hospital, Randwick, New South Wales, Australia
[e] Discipline of Psychiatry, University of Adelaide, South Australia, Australia
[f] Northern Adelaide Local Health Network, Australia
[g] Ramsay Health Care (SA) Mental Health, Australia
[h] Department of Neuroscience and Physiology, Upstate Medical University, Syracuse, NY 13210, USA

ARTICLE INFO

Article history:
Received 13 November 2018
Received in revised form 25 June 2019
Accepted 27 June 2019
Available online 8 July 2019

Keywords:
Schizophrenia
Schizoaffective disorder
Stress
Symptoms
Emotions
Stress signalling transcripts

ABSTRACT

Altered levels of stress-signalling transcripts have been identified in post-mortem brains of people with schizophrenia, and since stress effects may be expressed throughout the body, there should be similar changes in peripheral cells. However, the extent to which these markers are altered in peripheral white blood cells of people with schizophrenia is not known. Furthermore, how peripheral cortisol and stress-related mRNA are associated with negative symptom severity and emotional states in people with schizophrenia versus schizoaffective disorder has not been determined. Whole blood samples were collected from 86 patients with either schizophrenia or schizoaffective disorder (56 people with schizophrenia and 30 people with schizoaffective disorder), and 77 healthy controls. Total RNA was isolated, cDNA was synthesized, and stress-signalling mRNA levels (for NR3C1, FKBP5, FKBP4, PTGES3 and BAG1) were determined. Stress and symptom severity scores were measured by the Depression, Anxiety and Stress Scale, and the Positive and Negative Syndrome Scale, respectively. We found increased FKBP5 mRNA, $Z(156) = 2.5$, $p = 0.01$, decreased FKBP4 mRNA, $t(155) = 3.5$, $p \leq 0.001$, and decreased PTGES3 mRNA, $t(153) = 3.0$, $p \leq 0.01$, in schizophrenia and schizoaffective disorder cohorts combined compared to healthy controls. Stress-related peripheral mRNA levels were differentially correlated with negative emotional states and symptom severity in schizoaffective disorder (β's $= -0.45$–0.56, p's $= 0.05$–0.001) and schizophrenia (β's $= -0.34$–0.38, p's $= 0.04$–0.03), respectively. Therefore, molecules of the stress-signalling pathway appear to differentially contribute to clinical features of schizophrenia versus schizoaffective disorder.

1. Introduction

Schizophrenia is a severe mental disorder with heterogenous symptoms, cognitive and functional deficits and this variability can impede our understanding of the underlying biological mechanisms (Perkovic et al., 2017). A combination of both genetic and environmental factors are believed to pose a risk for developing schizophrenia (Nimgaonkar et al., 2017; Sullivan et al., 2003) and many deleterious environmental factors may act individually or in combination to increase stress. Epidemiological studies suggest that repeated stressful environments increase the risk for schizophrenia, with more childhood trauma experienced by people with schizophrenia (Agid et al., 1999; Arseneault et al., 2011; Betensky et al., 2008; Khashan et al., 2008). Stress is a known etiological factor for psychotic illnesses, as increased stress can precipitate onset, exacerbate symptom severity, and trigger relapse (Doering et al., 1998; Phillips et al., 2006; Yung et al., 2004). However, the cellular mechanism(s) by which stress acts to worsen psychosis is unknown.

The neural diathesis-stress model of schizophrenia proposes that there is a strong link between Hypothalamic Pituitary Adrenal (HPA) Axis activity and psychosis (Walker and Diforio, 1997). In humans, the primary glucocorticoid in the stress-signalling pathway is cortisol (Raymond et al., 2018) and people with

* Corresponding author at: Neuroscience Research Australia, Barker Street, Randwick, NSW 2031, Australia.
E-mail address: t.weickert@neura.edu.au (T.W. Weickert).
[1] These authors contributed equally.

https://doi.org/10.1016/j.schres.2019.06.026
0920-9964/

schizophrenia have alterations in regulation of this pathway. People with schizophrenia can experience either a heightened or a blunted secretion of cortisol when faced with psychological stress (Brenner et al., 2009; Brunelin et al., 2008). However, studies assessing absolute circulating levels of cortisol in people with major mental illness, including schizophrenia (Belvederi Murri et al., 2012; Muck-Seler et al., 2004; Watson et al., 2012) do not find a consistent change in cortisol levels, reviewed in Bradley and Dinan (2010), with many studies showing no difference in cortisol levels between people with psychotic illnesses relative to healthy controls (Mokrani et al., 2000; Ritsner et al., 2004). Conversely, other studies find higher (Meltzer et al., 2001; Muck-Seler et al., 1999), or lower (Phassouliotis et al., 2013; van Nimwegen et al., 2008), cortisol levels in people with schizophrenia compared to healthy controls. A recent meta-analysis revealed a significant, but small, increase in morning cortisol in people with schizophrenia compared to healthy controls (Girshkin et al., 2014). While differences in cortisol levels between people with psychotic illnesses and healthy controls have been inconsistent, a biological change in cortisol response may be altered through variation in the levels of glucocorticoid receptor (GR) or its binding proteins. Therefore, we hypothesised that variation in cortisol response downstream of cortisol itself may signal a differential stress response in people with schizophrenia.

It is important to understand the molecular mechanisms of the stress-signalling pathway to gain a better understanding of the stress-related pathophysiology in schizophrenia. NR3C1 is a gene that encodes the human GR (Bray and Cotton, 2003). Our lab has previously shown dysregulation of NR3C1 (GR), GR co-factors and chaperones in the dorsolateral prefrontal cortex (DLPFC) from post-mortem brains of people with schizophrenia (Sinclair et al., 2011; Sinclair et al., 2013). In fact, many studies have shown a transcriptional reduction in one of the main cortisol receptors, GR, in widespread cortical regions of the post-mortem brain of people with schizophrenia (Perlman et al., 2004; Sinclair et al., 2011; Webster et al., 2002). However, GR binds to many other cellular proteins to enable high affinity binding to cortisol, translocation to the nucleus and transcriptional activity. One GR binding protein, BAG1 mRNA encodes the Bag1 protein, which inhibits GR protein folding and has been found to be decreased in the DLPFC of people with schizophrenia (Sinclair et al., 2013). Additionally, a GR co-chaperone formed by the PTGES3 gene (p23) increases the assembly rate of the GR-hsp90 heterocomplex to increase GR affinity to cortisol (Morishima et al., 2000). Importantly, a prominent protein, FKBP5, acts to stabilize the GR heterocomplex in the high affinity state and also promote GR retention in the cytoplasm (Schiene-Fischer and Yu, 2001; Wochnik et al., 2005). When a ligand (e.g., cortisol) is bound to the GR heterocomplex, another GR binding protein FKBP52 (note similar in name to FKBP51), encoded by the FKBP4 gene, displaces the FKBP51 protein from the GR heterocomplex; thus, allowing the nuclear translocation of the GR heterocomplex into the nucleus to activate or repress target genes (Davies et al., 2002). See Fig. 1 for a schematic diagram of the GR-mediated stress signalling pathway (adapted from Sinclair et al., 2013). Both FKBP5 and PTGES3 mRNAs are increased in the DLPFC of people with schizophrenia (Sinclair et al., 2013) suggesting that the way the cortisol signal is processed in neural cells is altered in psychosis.

Dysregulation of important stress-related molecules in the brains of patients with schizophrenia can reveal a potential role of stress in psychotic illnesses and stress disorders. However, the extent to which these stress markers are also altered in peripheral blood cells in schizophrenia is unknown. Thus, in our present study, we aimed to determine the extent to which peripheral cortisol levels and peripheral expression levels of molecules of the stress-signalling pathway (in particular, NR3C1, FKBP5, FKBP4, PTGES3

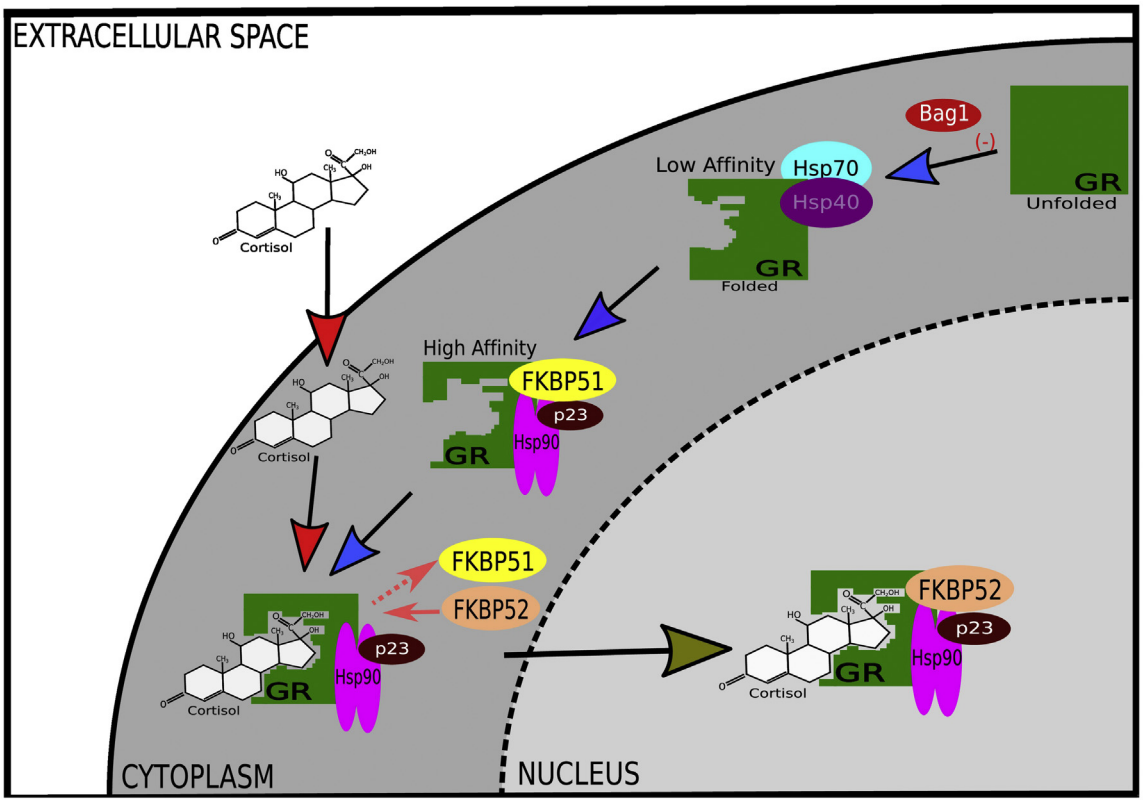

Fig. 1. Schematic diagram of the glucocorticoid receptor (GR) - mediated stress signalling pathway. GR is unable to bind cortisol when bound to Bag1, release of Bag1 allows GR to bind cortisol with low affinity while FKBP51 (encoded by FKBP5) and p23 (encoded by PTGES3) are involved in increasing GR affinity to cortisol by stabilizing the GR heterocomplex into a high affinity state. Furthermore, FKBP52 (encoded by FKBP4) dislocates FKBP51 and facilitates nuclear translocation of the cortisol-bound GR heterocomplex into the nucleus to activate or repress target genes. Thus, higher FKBP51 would promote GR retention in the cytoplasm rendering target genes less responsive to stress.

and BAG1) are changed in people with schizophrenia or schizoaffective disorder relative to healthy controls. We hypothesised that peripheral cortisol levels would be significantly different between patients and healthy controls and that there would be an increase in peripheral FKBP5 and PTGES3 mRNA expression and a decrease in NR3C1, FKBP4 and BAG1 mRNA expression in the peripheral blood of patients compared to healthy controls.

Since stress has also been linked to depression and anxiety (Khan and Khan, 2017), it is important to assess stress in the context of these symptoms. Salivary cortisol has been shown to positively correlate with positive, negative, general psychopathology and disorganised symptom severity in people who are at high-risk for psychosis (Walker et al., 2013). Additionally, despite the fact that stress modifies different symptoms of psychiatric illness, the extent to which the expression of stress-related genes may relate to emotional states and symptom severity in people with schizophrenia versus schizoaffective disorder has not been explored. In addition to having persistent psychotic symptoms of schizophrenia, people with schizoaffective disorder are characterised by periods of mania and/or depression. These symptoms involving mood, in particular euphoria, high energy levels and irritability (mania) and/or sadness and worthlessness (depression) are exclusive to people with schizoaffective disorder compared to people with schizophrenia (who conversely, often display flat/no affect) and they are frequently present (Abrams et al., 2008). Furthermore, the mood symptoms of a person diagnosed with schizoaffective disorder can be triggered by stressful life events, such as traumatic military experiences (Castine et al., 1998). Determining changes in the stress response at the molecular level in these two variants of schizophrenia that differ in the magnitude of psychotic and mood symptom profiles (Dell'Osso et al., 1993; Marneros et al., 1990) could suggest alternative treatment targets for emotional dysfunction versus psychotic and negative symptoms experienced by people with schizophrenia and schizoaffective disorder. The broader range of depressive, anxiety and stress symptoms in people with schizoaffective disorder may provide a better opportunity to determine the relationship between peripheral molecular markers of stress and behavioural measures of stress, independent from people with schizophrenia. Since age also influences stress related mRNA levels (Perlman et al., 2007; Weickert et al., 2016), age should be factored into any analysis of these measures.

Given that schizophrenia and schizoaffective disorder have been shown to differ on their range of emotional states, the current study further aimed to determine the extent to which age, peripheral cortisol, and expression of stress signalling molecules predict negative emotional states and psychotic symptom severity in schizophrenia relative to schizoaffective disorder. We hypothesised that age, peripheral cortisol, and stress-related peripheral mRNA levels would differentially be strong predictors of negative emotional states and symptom severity in people with schizoaffective disorder versus people with schizophrenia respectively.

2. Materials and methods

2.1. Participants

2.1.1. People with schizophrenia or schizoaffective disorder

Eighty-six participants (33 females and 53 males) with a diagnosis of schizophrenia (n = 56) or schizoaffective disorder (n = 30), between the ages of 20 and 51, who were receiving antipsychotic medication for at least one year, were recruited in response to a national television documentary and through the South Eastern Sydney and Illawarra Area Health Service and the Northern Adelaide Local Health Network Mental Health Service (Table 1). Diagnosis was determined via a Structured Clinical Interview for the Diagnostic and Statistical Manual IV-TR Axis I Disorders (SCID) (First et al., 2007) administered by either a psychiatrist or psychologist and confirmed by an independent psychiatrist. Exclusion criteria included patients who had an additional Axis 1 psychiatric diagnosis other than schizophrenia or schizoaffective disorder, a history of substance abuse/dependence within the past five years, head injuries with a loss of consciousness, seizures, central nervous system infection, uncontrolled diabetes or hypertension, mental retardation, and for women, those who were pregnant.

2.1.2. Healthy controls

For comparative purposes, 77 healthy controls (38 females and 39 males), between the ages of 20 and 50, were recruited from

Table 1
Demographics and clinical characteristics of healthy controls versus people with schizophrenia/schizoaffective disorder.

	Healthy controls (n = 77)	Patients (n = 86)	df	t/Z/χ^2 value	p-Value
Age (years)	31.7 (8.5)	35.8 (8.3)	161	−3.1	0.002*
Education (years)	14.5 (2.3)	12.6 (2.5)	161	5.1	<0.001*
WAIS-III					
Estimated full scale IQ	107.5 (15.0)	91.5 (12.7)	161	7.4	<0.001*
WTAR					
Estimated premorbid IQ	108.2 (8.8)	103.4 (8.1)	161	3.6	<0.001*
Sex (M/F)	39/38	53/33	1.0	2.0	0.16
Ethnicity (total)			2.0	0.6	0.74
Caucasian	62	73			
Asian	11	10			
Other	4	3			
DASS					
Depression	3.7 (5.7)	12.1 (9.7)	158	−6.1	<0.001*
Anxiety	3.0 (4.3)	10.2 (7.7)	158	−6.7	<0.001*
Stress	6.7 (8.1)	14.6 (9.5)	158	−5.7	<0.001*
Schizoaffective/schizophrenia	–	30/56	–	–	–
Age of illness onset	–	22.9 (5.8)	–	–	–
Illness duration	–	12.9 (7.5)	–	–	–
Antipsychotic (CPZ) dose (mg/day)	–	554.7 (463.4)	–	–	–
Imipramine equivalent dose (mg/day)		194.0 (146.3)			
PANSS					
Positive	–	15.2 (4.4)	–	–	–
Negative	–	14.5 (6.2)	–	–	–
General	–	30.8 (8.8)	–	–	–
Total	–	60.5 (16.4)	–	–	–

Note. – = not applicable; WAIS-III = Wechsler Adult Intelligence Scale-Third Edition; WTAR = Wechsler Test of Adult Reading; DASS = Depression Anxiety Stress Scales; CPZ = mean daily Chlorpromazine Equivalence dose; PANSS = Positive and Negative Syndrome Scale; All data shown in brackets are standard deviations.
* $p \leq 0.05$.

Sydney, and Adelaide, Australia, through advertisements placed throughout the community. Healthy adults who had a personal history of or a first-degree relative with a DSM-IV Axis I psychiatric diagnosis, history of substance abuse or dependence within the past five years, head injuries with a loss of consciousness, seizures, central nervous system infection, uncontrolled diabetes or hypertension, mental retardation and for women, those who were pregnant were excluded.

All participants provided informed, written consent prior to commencing the study, and the protocol was approved by the University of New South Wales (07/121 and 09/187), South Eastern Sydney and Illawarra Area Health Service (07-259) Human Research Ethics Committees (Sydney, New South Wales), and the Queen Elizabeth Hospital Ethics and Human Research Committee (2010188) (Adelaide, South Australia).

2.2. Cognitive assessments

All participants were administered a four subtest version of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) (Wechsler, 1997), including the Arithmetic, Similarities, Picture Completion and Digit Symbol Substitution subtests, which provided an estimate of current intellectual ability. The Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001) was used as a "hold" measure of intellect (premorbid IQ).

2.3. Whole blood collection and processing

Whole blood samples were collected in a quiet medical exam room via venepuncture by a phlebotomist, using ACD-B tubes and serum separating tubes (Interpath), from 86 people with schizophrenia and 77 healthy controls, between 9 and 11 AM. Samples (8–9 ml volume) were centrifuged at 400*g*, supernatant was removed and the RNA pellet was resuspended in 1 ml of cell protect reagent in 15 ml falcon tubes and stored at −80 °C until the time of assay.

2.4. Cortisol assay

Peripheral cortisol levels were determined from serum (blood collection as described above) using an Elecsys Cortisol I assay by the South Eastern Area Laboratory Services of NSW Health Pathology.

2.5. RNA extraction

Total RNA was isolated from peripheral blood cells using trizol extraction and RNA concentration was quantified using a ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA integrity was assessed using a high-resolution capillary electrophoresis (Agilent Bioanalyzer 2100, Agilent Technologies, Palo Alto, CA, USA). See Supplemental Methods for more details of the RNA extraction procedure.

2.6. cDNA synthesis

Total RNA from each sample was reversed transcribed to cDNA using SuperScript III First-Strand cDNA synthesis reaction and random hexamers, following the manufacturer's protocol (Invitrogen). The reaction was repeated without the addition of a reverse transcriptase as a negative control.

2.7. Quantitative real-time PCR

Expression of the five genes of interest (NR3C1, FKBP5, FKBP4, PTGES3, BAG1) was determined using a Fluidigm mRNA expression assay by the Ramaciotti Centre for Genomics at the University of New South Wales. Taqman Gene Expression probes selected for each of the stress-related genes were: NR3C1 (Hs00230813_m1), FKBP5 (Hs01561006_m1), FKBP4 (Hs00427038_g1), PTGES3 (Hs00832847_gH) and BAG1 (Hs00185390_m1). Expression levels of each gene were normalised to the geometric mean of four housekeeper genes (B2M, GAPDH, TBP and UBC), which were run in the same multiplex assay and did not differ between patient and control groups. The Taqman probes used to measure housekeeper genes were: B2M (Hs00984230_m1), GAPDH (Hs99999905_m1), TBP (Hs00427620_m1), UBC (Hs00824723_m1). Peripheral mRNA expression levels were also normalised between plates using an internal control (made from a mixed sample of both patients and controls). Outliers were removed if values exceeded plus or minus two standard deviations from the means of each measure (controls: 2–4 outliers/target, patients: 1–4 outliers/target).

2.8. Stress and symptom severity scores

All patients were interviewed for current symptom severity by a psychologist or psychometrician who had training in administration and scoring of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Depression, anxiety and stress scores were measured in patients and controls using the Depression, Anxiety and Stress Scale (DASS) self-report questionnaire (Lovibond and Lovibond, 1995).

2.9. Statistical analyses

Demographic variables were compared between all patients and healthy controls using independent samples *t*-tests, Mann Whitney *U*-Tests or chi square tests, as appropriate. Independent samples *t*-tests or Mann Whitney *U*-Tests were performed, as appropriate, to assess differences in peripheral cortisol levels and peripheral molecules of the stress-signalling pathway between healthy controls and patients. Data that were not normally distributed were log-transformed to obtain normality. Non-parametric tests were used for data that did not achieve a normal distribution after log-transformation.

Demographic variables were compared between people with schizophrenia and people with schizoaffective disorder using independent samples *t*-tests, Mann Whitney U-Tests or chi square tests, as appropriate. A one-way analysis of variance (ANOVA) or Kruskal-Wallis H test were performed, as appropriate, to assess differences in peripheral cortisol levels and peripheral molecules of the stress-signalling pathway among healthy controls, people with schizophrenia and people with schizoaffective disorder, separately.

Separate multiple linear backward stepwise regressions were performed to determine the relationship of age, peripheral cortisol and stress-related peripheral mRNA levels to depression, anxiety and stress scores separately in the 3 diagnostic groups: healthy controls, people with schizophrenia and people with schizoaffective disorder. Predictor variables included age, peripheral cortisol levels and peripheral NR3C1, FKBP5, FKBP4, BAG1 and PTGES3 mRNA levels. Dependent variables included depression, anxiety and stress scale scores.

Likewise, separate multiple linear backward stepwise regressions were performed to assess the relationship of age, peripheral cortisol and stress-related peripheral mRNA levels to PANSS positive, negative and general symptom severity scores in people with schizophrenia and schizoaffective disorder, separately. Predictor variables included age, peripheral cortisol levels and peripheral NR3C1, FKBP5, FKBP4, BAG1 and PTGES3 mRNA levels. Dependent variables included PANSS positive, negative and general symptom severity scores.

To examine the potential influence of antipsychotics, antipsychotic dosages were converted to mean daily chlorpromazine equivalent (CPZ) dose using standard guidelines (Leucht et al., 2003) and Spearman correlations between mean daily CPZ

equivalent dose and cortisol levels were performed in people with schizophrenia and people with schizoaffective disorder, separately. Separate Spearman correlations between age and stress-related peripheral mRNA levels were performed separately in healthy controls, people with schizoaffective disorder and people with schizophrenia to identify any specific effect of age on stress related molecules. Pearson correlations were also performed to determine correlations among the stress signalling mRNA variables separately in all patients and controls.

3. Results

3.1. Demographics

Demographic and clinical comparisons between healthy controls and all patients are presented in Table 1. Healthy controls were significantly younger (percent difference = 12%) and, as expected, healthy controls had significantly more education, higher current IQ estimates and less depression, anxiety and stress compared to people with schizophrenia. Patients and healthy controls did not differ significantly on sex and ethnicity ratios. Based on the PANSS scores, symptom severity of the patients with schizophrenia was mild to moderate.

See Table 2 for demographic and clinical comparisons between people with schizophrenia and schizoaffective disorder. Patients with schizoaffective disorder showed significantly greater illness duration and DASS anxiety and stress scores relative to patients with schizophrenia. Conversely, the patients with schizophrenia displayed significantly greater PANSS negative symptoms and total scores relative to patients with schizoaffective disorder. There were no other significant differences between the patient diagnostic groups.

Table 2
Demographics and clinical characteristics of people with schizoaffective disorder versus people with schizophrenia.

	Schizoaffective disorder (n = 30)	Schizophrenia (n = 56)	df	t/Z/χ^2 value	p-Value
Age (years)	38.1 (7.4)	34.6 (8.6)	84	−1.9	0.06
Education (years)	12.9 (2.9)	12.5 (2.2)	84	−0.4	0.69
WAIS-III					
Estimated full scale IQ	94.6 (13.5)	89.9 (12.1)	84	−1.6	0.11
WTAR					
Estimated premorbid IQ	104.4 (7.6)	102.8 (8.3)	84	−0.87	0.39
Sex (M/F)	15/15	38/18	1.0	2.6	0.11
Ethnicity (total)			2.0	1.6	0.45
Caucasian	24	49			
Asian	4	6			
Other	2	1			
DASS					
Depression	14.4 (10.9)	10.9 (8.8)	84	−1.6	0.12
Anxiety	12.7 (7.0)	8.8 (7.8)	84	−2.3	0.02*
Stress	17.5 (9.8)	13.0 (9.0)	84	−2.2	0.03*
Age of illness onset	23.2 (5.5)	22.8 (5.9)	84	−0.3	0.79
Illness duration	15.3 (6.9)	11.7 (7.6)	84	−2.2	0.03*
Antipsychotic (CPZ) dose (mg/day)	549.3 (385.9)	557.7 (503.3)	84	0.1	0.94
Imipramine equivalent dose (mg/day)	260.4 (179.4)	147.5 (103.8)	15	−1.6	0.12
PANSS					
Positive	14.1 (4.4)	15.8 (4.4)	84	1.7	0.09
Negative	11.3 (4.2)	16.2 (6.4)	84	−3.7	<0.001*
General	29.1 (6.4)	31.7 (9.7)	84	−1.0	0.30
Total	54.5 (11.4)	63.7 (17.8)	84	−2.4	0.02*

Note. − = not applicable; WAIS-III = Wechsler Adult Intelligence Scale-Third Edition; WTAR = Wechsler Test of Adult Reading; DASS = Depression Anxiety Stress Scales; CPZ = mean daily Chlorpromazine Equivalence dose; PANSS = Positive and Negative Syndrome Scale; All data shown in brackets are standard deviations.
* $p \leq 0.05$.

3.2. No diagnostic differences in peripheral cortisol levels

We found no significant difference in peripheral cortisol levels between patients (mean = 299.4 nmol/l, SD = 112.6) and healthy controls (mean = 298.1 nmol/l, SD = 108.5), Z(154) = −0.04, p = 0.97. Likewise, based on a Kruskal-Wallis H test, no significant differences in peripheral cortisol levels were detected among healthy controls (mean = 298.1 nmol/L, SD = 108.5), people with schizophrenia (mean = 311.2 nmol/l, SD = 114.1) and people with schizoaffective disorder (mean = 278.4 nmol/l, SD = 108.7), χ^2 (2) = 1.90, p = 0.39.

3.3. Stress-related molecular markers differ between patients and healthy controls

We found a significant increase in peripheral mRNA levels of FKBP5, Z(156) = 2.5, p = 0.01, and significant decreases in peripheral PTGES3, t(153) = 3.0, $p \leq 0.01$, and FKBP4, t(155) = 3.5, $p \leq 0.001$, in the combined schizophrenia/schizoaffective disorder group compared to healthy controls (see Fig. 2). In contrast, we did not detect diagnostic differences in peripheral NR3C1, t(157) = 1.1, p = 0.29, or BAG1, t(156) = 0.63, p = 0.53, mRNA levels between patients and healthy controls. See Supplementary Results for effects of sex differences in stress-related peripheral mRNA levels. See Supplementary Table 1 for the correlations among stress-signalling mRNA levels in healthy controls and people with schizophrenia/schizoaffective disorder.

3.4. Stress-related mRNAs differ among healthy controls and people with schizophrenia, but not people with schizoaffective disorder

Peripheral FKBP5, PTGES3 and FKBP4 mRNA levels differed significantly between people with schizophrenia and healthy controls, but not between people with schizoaffective disorder and healthy controls (see Supplementary Fig. 1). Peripheral NR3C1 and BAG1 mRNA did not significantly differ among people with schizophrenia, schizoaffective disorder and healthy controls (see Supplementary Fig. 1).

3.5. Stress-related molecular markers contribute to depression, anxiety and stress levels in those with schizoaffective disorder

In people with schizoaffective disorder, peripheral FKBP5 mRNA positively and significantly predicted depression, anxiety and stress scores (see Table 3). PTGES3 mRNA positively and significantly predicted anxiety and stress, but not depression (see Table 3) in people with schizoaffective disorder. NR3C1 mRNA positively and significantly predicted anxiety (see Table 3), and in contrast, FKBP4 mRNA significantly and inversely predicted anxiety in those with schizoaffective disorder (see Table 3). Unlike what was shown in people with schizoaffective disorder, in both healthy controls and people with schizophrenia, peripheral stress-related mRNA levels did not significantly predict depression, anxiety or stress scale scores.

3.6. Stress-related molecular markers contribute to symptom severity in both people with schizophrenia and schizoaffective disorder

In people with schizoaffective disorder, NR3C1 mRNA positively and significantly predicted PANSS positive score ($\beta = 0.56$, p = 0.002, % of unique variance explained = 30.1%), and PTGES3 mRNA showed a trend towards predicting PANSS positive symptom severity score ($\beta = 0.30$, p = 0.07, % of unique variance explained = 8.8%). No other predictor variables from the model contributed to PANSS positive, negative or general psychopathology scores in schizoaffective disorder.

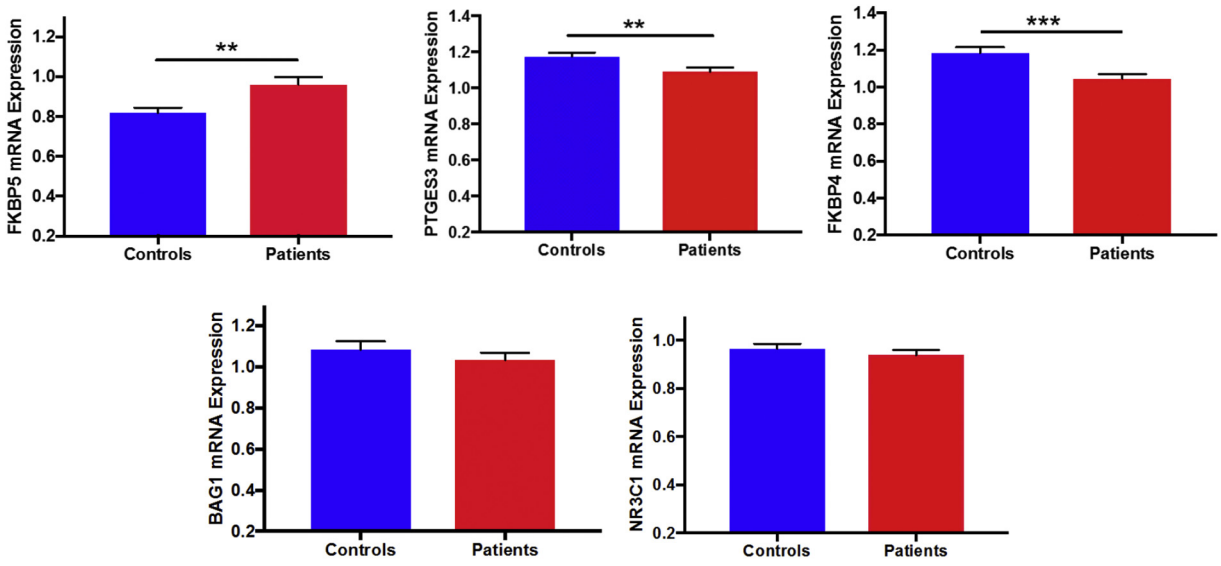

Fig. 2. Differences of stress- related peripheral mRNA expression levels in healthy controls versus people with schizophrenia/ schizoaffective disorder. ** $p \leq 0.01$; *** $p \leq 0.001$; Error bars show standard error of mean (SEM).

In people with schizophrenia, FKBP5 mRNA positively and significantly predicted PANSS positive symptom severity score (see Table 4) and FKBP5 mRNA showed a trend towards predicting PANSS negative symptom severity score (see Table 4). FKBP4 mRNA positively and significantly predicted PANSS negative and PANSS general symptom severity scores (see Table 4). Peripheral PTGES3 mRNA levels inversely predicted PANSS negative and PANSS general symptom severity score (see Table 4).

3.7. Consideration of age and antipsychotics on molecular stress measures

There were no correlations between cortisol levels and mean daily CPZ equivalent dose in people with schizoaffective disorder ($r = -0.03$, $p = 0.87$) or people with schizophrenia ($r = 0.03$, $p = 0.85$). Age showed a weak, significant inverse correlation with FKBP5 ($r = -0.23$, $p = 0.05$), and NR3C1 ($r = -0.23$, $p = 0.05$) mRNA expression in healthy controls. There were no strong, significant correlations between age and FKBP5 mRNA in schizoaffective disorder ($r = -0.15$, $p = 0.44$) or schizophrenia ($r = -0.08$, $p = 0.57$); nor between age and NR3C1 mRNA in schizoaffective disorder ($r = -0.06$, $p = 0.76$) or schizophrenia ($r = -0.01$, $p = 0.96$). There was a weak, significant, inverse correlation between age and FKBP4 mRNA in people with schizophrenia ($r = -0.28$, $p = 0.04$), but no such relationship in healthy controls ($r = -0.14$, $p = 0.24$) or people with schizoaffective disorder ($r = -0.17$, $p = 0.38$). There were no strong, significant correlations between age and PTGES3 or BAG1 in controls ($r = -0.05$, $p = 0.70$; $r = -0.05$, $p = 0.69$), schizoaffective disorder ($r = 0.18$, $p = 0.36$; $r = 0.15$, $p = 0.42$) or schizophrenia ($r = -0.09$, $p = 0.52$; $r = 0.10$, $p = 0.48$), respectively.

Table 3
Multiple linear backward stepwise regression analyses assessing the effects of cortisol and peripheral NR3C1, FKBP5, FKBP4, PTGES3 and BAG1 mRNA levels on depression, anxiety and stress scale scores in people with schizoaffective disorder.

		Schizoaffective disorder				
		R	p	% variance explained	*β*	p
Depression		0.56	0.001			
	FKBP5			31.8	0.56	0.001
Anxiety		0.68	0.003			
	NR3C1			11.2	0.34	0.03
	FKBP5			16.2	0.42	0.01
	FKBP4			14.3	−0.45	0.02
	PTGES3			20.3	0.54	0.006
Stress		0.63	0.005			
	FKBP5			25.1	0.52	0.004
	FKBP4			8.3	−0.34	0.08
	PTGES3			9.9	0.37	0.05

4. Discussion

Overall, we found that the peripheral molecular markers of stress responsivity are changed in people with schizophrenia and seem to be biased towards positioning these patients to be less responsive to increases in cortisol in spite of similar circulating morning cortisol levels. Importantly, we found that the molecular measures taken from within white blood cells correlate with the indices of stress and symptom severity perceived or exhibited by these patients. This suggests that the increased stress, anxiety and symptoms perceived/ expressed by patients with major mental illness are intimately linked to the biological response of cells to cortisol.

The primary findings of this study were: 1) significant alterations in the peripheral mRNA levels of key GR cofactors/chaperones (i.e., FKBP5, FKBP4 and PTGES3) in patients with schizophrenia compared to healthy controls, 2) significant ability of stress-related peripheral mRNA markers to predict depression, anxiety and stress in people with schizoaffective disorder, and 3)

Table 4
Multiple linear backward stepwise regression analyses assessing the effects of cortisol and peripheral NR3C1, FKBP5, FKBP4, PTGES3 and BAG1 mRNA levels on PANSS positive, negative and general symptom severity scores in people with schizophrenia.

		Schizophrenia				
		R	p	% variance explained	*β*	p
PANSS positive		0.32	0.03			
	FKBP5			10.0	0.32	0.03
PANSS negative		0.46	0.02			
	FKBP5			6.0	−0.25	0.08
	FKBP4			10.1	0.38	0.03
	PTGES3			8.5	−0.34	0.04
PANSS general		0.36	0.05			
	FKBP4			9.3	0.36	0.04
	PTGES3			10.2	−0.37	0.03

stress-related peripheral mRNA levels significantly predicting symptom severity in both schizophrenia and schizoaffective disorder. Contrary to our hypothesis, peripheral cortisol, the cortisol receptor, GR (NR3C1), and the GR co-factor (BAG1) mRNAs did not differ significantly between patients versus healthy controls. Additionally, outside of the context of having a major mental illness, we find that age, peripheral stress-related molecules and circulating cortisol did not predict depression, anxiety and stress in healthy controls.

We found no significant difference in peripheral cortisol levels among people with schizophrenia, people with schizoaffective disorder and healthy controls. Some studies (Mokrani et al., 2000; Ritsner et al., 2004) also failed to show a significant difference in peripheral cortisol levels between patients with schizophrenia and controls. This may be due to a number of factors that influence cortisol rhythm, such as time of morning the blood was taken relative to typical wake times, physical activity and number of hours slept (Ice, 2005). Thus, the constantly fluctuating diurnal rhythm of cortisol suggests that cortisol may need to be measured over a period of time, as opposed to a single measurement. However, our results suggest that changes in cortisol processing in schizophrenia and schizoaffective disorder occur downstream of cortisol itself.

In support of our hypothesis, we found a significant increase in peripheral FKBP5 mRNA in the combined group of patients with schizophrenia and schizoaffective disorder. This result is similar to our post-mortem brain study in which we found an increase in FKBP5 mRNA expression in the DLPFC of people with schizophrenia (Sinclair et al., 2013). The possible parallel up-regulation of FKBP5 mRNA in both the blood and post mortem brain (Sinclair et al., 2013) of people with schizophrenia, compared to healthy controls, may be supportive of using FKBP5 as a blood-based biomarker for mental illness, as changes in peripheral FKBP5 seems to reflect the pattern found in the brain of patients (Harris et al., 2012). Increase in FKBP5 mRNA expression in the brains of male adult mice using stress paradigms has also been reported (Scharf et al., 2011) demonstrating that stress leads to elevated FKBP5 in an experimental model and supporting a link among stress, FKBP5 and symptoms in schizophrenia. Others have also shown elevated FKBP5 protein in the cytoplasm of peripheral blood mononuclear cells in major depressive disorder compared to healthy controls (Lukic et al., 2015) and increased FKBP5 mRNA expression in the prefrontal cortex of people with bipolar disorder compared to healthy controls (Sinclair et al., 2013). Thus, an increase in FKBP5 mRNA and/or protein may be prevalent across psychiatric diagnoses. Our present findings support the role of FKBP5 in schizophrenia and, in conjunction with previous findings, major mental illness in general. Increased FKBP5 mRNA and protein found in animal models and human studies suggest that this is a robust feature of psychiatric illness.

As predicted, we found that FKBP4 mRNA was decreased in people with schizophrenia and schizoaffective disorder, which has not been previously shown in the peripheral blood of people with schizophrenia. Since FKBP52 (the protein product of the FKBP4 gene) dislodges FKBP51 (the protein product of the FKBP5 gene) from the cortisol-bound GR complex; the actions of these two proteins are opposing (Davies et al., 2002). Thus, the respective changes in the levels of peripheral FKBP5 and FKBP4 mRNAs, suggests that people with schizophrenia may have an overactive stress response pathway possibly resulting in a compensatory response to block cortisol action that may result in a blunted negative feedback ability. Likewise, reduced levels of peripheral PTGES3 mRNA, and subsequently the functional p23 protein, in patients with schizophrenia suggests a further inability or disruption for normal glucocorticoid signalling; thus, causing an overall dysregulation of the cellular stress signalling response that has some similarities but also some differences as to what is found in the brains of people with schizophrenia.

Unlike what was found in the post mortem brain of people with schizophrenia where PTGES3 mRNA was increased (Sinclair et al., 2013), peripheral PTGES3 mRNA levels were decreased in people with schizophrenia compared to healthy controls. Therefore, this challenges an overreliance on the use of peripheral gene expression as signalling the direction of significant changes in human brain (Sullivan et al., 2006), and confirming that some markers found in the brain may fail to be found in the peripheral blood (Lai et al., 2016). This discrepancy demonstrates that there may be brain specific transcriptional changes in stress response pathway members in schizophrenia.

Regarding correlations among the stress signalling mRNA levels, we found moderately strong, positive correlations between the GR factor enabling nuclear translocation (FKBP52 encoded by the FKBP4 gene) and the mRNA for p23 (PTGES3) suggesting that p23 is co-regulated with or positively regulated by the FKBP52-GR complex in both healthy and disease states. However, that was not the case for BAG1 and GR mRNAs. While we found marginal, yet significant, positive correlations with BAG1 and NR3C1 mRNAs and FKBP52 in healthy controls, there were no strong, significant correlations among these factors in patients. This suggests that the weak positive feedback loop may be dysregulated in psychotic illness. Future studies should test for the existence of glucocorticoid receptor response elements in the promoter region of these genes (Sasse et al., 2015).

Interestingly, we found that FKBP5 predicted depression, anxiety and stress in schizoaffective disorder and PANSS positive symptom severity in people with schizophrenia. This is supportive of previous studies showing a relationship of the FKBP5 polymorphism with childhood abuse and depression, specifically in post-traumatic stress disorder (Binder et al., 2008) and major depressive disorder (Rao et al., 2016). FKBP5 gene polymorphisms increased the risk for major depressive disorder, which is characterised by emotional states of depression, anxiety and stress (Szczepankiewicz et al., 2014).

We found an inverse relationship between peripheral FKBP4 mRNA and anxiety in schizoaffective disorder and a positive relationship of FKBP4 with PANSS negative and general symptom severity scores in people with schizophrenia. Thus, alterations in same molecule appears to influence these two disorders with opposite valence, where higher levels of FKBP4 appear to be related to better affective symptoms (i.e., lower scores) in schizoaffective disorder; whereas higher levels are related to more psychopathology in those with schizophrenia. Similarly, peripheral PTGES3 positively predicted stress and anxiety in schizoaffective disorder, but there was an inverse relationship between PTGES3 and PANSS negative and general symptom severity in schizophrenia. Finally, peripheral NR3C1 mRNA positively predicted anxiety and PANSS positive symptom severity in schizoaffective disorder. Thus, the effects of stress regulating molecules are largely prominent in relationship to the negative emotional component of schizoaffective disorder and, conversely, they are prominent in relationship to symptom severity in people with schizophrenia and may modify symptoms in distinct ways. Given that these stress-related molecules differentially influence unique and specific behavioural aspects of these conditions, these results support the notion that schizophrenia and schizoaffective disorder have some distinct biological aspects. Our results provide biological support for a distinct diagnosis of schizoaffective disorder as opposed to a sole diagnosis of schizophrenia consistent with the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (Malaspina et al., 2013).

Given the role of these stress-related molecules in the pathophysiology of schizophrenia, the importance of these findings

should be put in context. Remarkably, the large amount of variance accounted for by these key stress-related molecules taken together, especially in relation to anxiety in people with schizoaffective disorder (i.e., 62% of the unique variance explained), indicates the importance of these stress-related molecules to the specific clinical phenotypes. Therefore, it may be useful to assess specific molecules within the stress-signalling pathway as potential biomarkers for the clinical phenotypes exhibited based on these subtypes of the illness. Although the peripheral stress-related molecules showed significant differences only between schizophrenia and healthy controls, the relationships of these stress-related molecules to negative emotions in schizoaffective disorder suggests that these molecules have downstream effects on behaviour even in schizoaffective disorder alone. While the results may be a consequence of greater illness severity, this explanation would not be supported on the basis of the PANSS scores in our sample of patients that shows significantly worse symptom severity in the patients with schizophrenia relative to patients with schizoaffective disorder.

Our study is potentially limited in that the sample size for schizophrenia and schizoaffective groups were not balanced. An imbalance in sample size between comparison groups may yield no significant difference between groups due to a larger variance in the small sample size group. The small sample size would be expected to have increased variance and make group differences more difficult to detect. However, in our study, the smaller schizoaffective disorder group did not have a considerably greater variance as compared to the control group or the schizophrenia group for any measure. Additionally, the schizoaffective group did not show as great of a mean difference from controls as compared to the schizophrenia group, indicating that this smaller sample was intermediate in relation to the variance which would not make differences among the groups more difficult to detect. In addition, we may be underpowered to find an effect on differential gene expression that exists only in people with schizoaffective disorder. However, given that we found statistically significant correlations in our sample with the lowest number of subjects, this suggests that we were not underpowered for this analysis. Moreover, our study is also limited in that it is restricted to analysis of one hormone level and only several mRNAs. Future studies should investigate the relative expression level of the functional protein in relation to the stress-signalling molecules. In addition, the study of DNA to investigate epigenetic markers that may be coded by early life adversity may be beneficial in predicting the differential set points of stress-related molecules found in our study. Also, due to the heterogeneity of the illness and the effect of stress on inflammation, it seems advisable to cluster patients into high and low inflammation groups to further explore stress related changes in these subgroups of schizophrenia (Fillman et al., 2014; Fillman et al., 2016).

In conclusion, differential dysregulation of the stress-signalling pathway in peripheral blood markers is evident in schizophrenia versus schizoaffective disorder, which is generally consistent with our previous results in the post-mortem brain of people with schizophrenia. Molecular changes of stress-related mRNA in blood were differentially related to perception of emotional state and stress in people with schizoaffective disorder and symptom severity in schizophrenia and schizoaffective disorder. Distinct molecules of the stress-signalling pathway, such as FKBP5, PTGES3 and FKBP4 mRNA, may serve as a useful theranostic biomarker for clinical features of people with schizophrenia or schizoaffective disorder (Weickert et al., 2013).

Contributors

CHL performed statistical analyses, prepared the first draft of and edited the manuscript; DS edited the manuscript; MOD assessed patients and edited manuscript; CG assessed patients and edited manuscript; DL assessed patients and edited manuscript; CSW designed the study and edited the manuscript; TWW designed the study, wrote the protocol and edited the manuscript. All authors have contributed to and approved the final manuscript.

Declaration of Competing Interest

CSW is on an advisory board for Lundbeck, Australia Pty Ltd. and has received funding for a project from Astellas Pharma Inc., Japan. All authors declare that they have no competing financial interests in relation to the work described in this report.

Acknowledgements

We would like to thank Ans Vercammen, Ruth Wells, Loretta Moore, Nicholas Vella, and Alice Rothwell for assisting with participant recruitment, administering and scoring neuropsychological and symptom assessments, as well as collecting and processing blood.

Funding

This work was funded by the New South Wales Ministry of Health, Office of Health and Medical Research, Australia. This work was also supported by the National Health and Medical Research Council (NHMRC) of Australia Project Grant (# 568807), the Pratt Foundation, Ramsay Health Care, the Viertel Charitable Foundation and the Australian Schizophrenia Research Bank (ASRB), which is supported by the NHMRC of Australia. CSW is a recipient of a National Health and Medical Research Council (Australia) Principal Research Fellowship (PRF) (#1117079). CHL was generously supported by an Australian Postgraduate Award and Neuroscience Research Australia Top-Up Scholarship.

Appendix A. Supplementary data

Supplementary material to this article can be found online at https://doi.org/10.1016/j.schres.2019.06.026.

References

Abrams, D.J., Rojas, D.C., Arciniegas, D.B., 2008. Is schizoaffective disorder a distinct categorical diagnosis? A critical review of the literature. Neuropsychiatr. Dis. Treat. 4 (6), 1089–1109.
Agid, O., et al., 1999. Environment and vulnerability to major psychiatric illness: a case control study of early parental loss in major depression, bipolar disorder and schizophrenia. Mol. Psychiatry 4 (2), 163–172.
Arseneault, L., et al., 2011. Childhood trauma and children's emerging psychotic symptoms: a genetically sensitive longitudinal cohort study. Am. J. Psychiatry 168 (1), 65–72.
Belvederi Murri, M., et al., 2012. Hypothalamic-pituitary-adrenal axis and clinical symptoms in first-episode psychosis. Psychoneuroendocrinology 37 (5), 629–644.
Betensky, J.D., et al., 2008. Patterns of stress in schizophrenia. Psychiatry Res. 160 (1), 38–46.
Binder, E.B., et al., 2008. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. Jama 299 (11), 1291–1305.
Bradley, A.J., Dinan, T.G., 2010. A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. J. Psychopharmacol. 24 (4), 91–118.
Bray, P.J., Cotton, R.G., 2003. Variations of the human glucocorticoid receptor gene (NR3C1): pathological and in vitro mutations and polymorphisms. Hum. Mutat. 21 (6), 557–568.
Brenner, K., et al., 2009. Cortisol response to a psychosocial stressor in schizophrenia: blunted, delayed, or normal? Psychoneuroendocrinology 34 (6), 859–868.
Brunelin, J., et al., 2008. Effects of acute metabolic stress on the dopaminergic and pituitary-adrenal axis activity in patients with schizophrenia, their unaffected siblings and controls. Schizophr. Res. 100 (1–3), 206–211.
Castine, M.R., Meador-Woodruff, J.H., Dalack, G.W., 1998. The role of life events in onset and recurrent episodes of schizophrenia and schizoaffective disorder.

J. Psychiatr. Res. 32 (5), 283–288.
Davies, T.H., Ning, Y.M., Sanchez, E.R., 2002. A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J. Biol. Chem. 277 (7), 4597–4600.
Dell'Osso, L., et al., 1993. Psychotic and nonpsychotic bipolar mixed states: comparisons with manic and schizoaffective disorders. Eur. Arch. Psychiatry Clin. Neurosci. 243 (2), 75–81.
Doering, S., et al., 1998. Predictors of relapse and rehospitalization in schizophrenia and schizoaffective disorder. Schizophr. Bull. 24 (1), 87–98.
Fillman, S.G., et al., 2014. Markers of inflammation and stress distinguish subsets of individuals with schizophrenia and bipolar disorder. Transl. Psychiatry 4.
Fillman, S.G., et al., 2016. Elevated peripheral cytokines characterize a subgroup of people with schizophrenia displaying poor verbal fluency and reduced Broca's area volume. Mol. Psychiatry 21 (8), 1090–1098.
First, M.B., et al., 2007. Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Patient Edition (SCID-I/P). New York Biometric Research Department, New York State Psychiatric Institute.
Girshkin, L., et al., 2014. Morning cortisol levels in schizophrenia and bipolar disorder: a meta-analysis. Psychoneuroendocrinology 49, 187–206.
Harris, L.W., et al., 2012. Comparison of peripheral and central schizophrenia biomarker profiles. PLoS One 7 (10), e46368.
Ice, G.H., 2005. Factors influencing cortisol level and slope among community dwelling older adults in Minnesota. J. Cross. Cult. Gerontol. 20 (2), 91–108.
Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.
Khan, S., Khan, R.A., 2017. Chronic stress leads to anxiety and depression. Ann. Psychiatry Ment. Health 5 (1), 1091.
Khashan, A.S., et al., 2008. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. Arch. Gen. Psychiatry 65 (2), 146–152.
Lai, C.Y., et al., 2016. Biomarkers in schizophrenia: a focus on blood based diagnostics and theranostics. World J. Psychiatry. 6 (1), 102–117.
Leucht, S., et al., 2003. New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. Lancet 361 (9369), 1581–1589.
Lovibond, S., Lovibond, P., 1995. Manual for the Depression Anxiety Stress Scale, 2nd edition. Psychology Foundation, Sydney, Australia.
Lukic, I., et al., 2015. Accumulation of cytoplasmic glucocorticoid receptor is related to elevation of FKBP5 in lymphocytes of depressed patients. J. Mol. Neurosci. 55 (4), 951–958.
Malaspina, D., et al., 2013. Schizoaffective disorder in the DSM-5. Schizophr. Res. 150 (1), 21–25.
Marneros, A., Deister, A., Rohde, A., 1990. Psychopathological and social status of patients with affective, schizophrenic and schizoaffective disorders after long-term course. Acta Psychiatr. Scand. 82 (5), 352–358.
Meltzer, H.Y., Lee, M.A., Jayathilake, K., 2001. The blunted plasma cortisol response to apomorphine and its relationship to treatment response in patients with schizophrenia. Neuropsychopharmacology 24 (3), 278–290.
Mokrani, M., et al., 2000. Multihormonal responses to clonidine in patients with affective and psychotic symptoms. Psychoneuroendocrinology 25 (7), 741–752.
Morishima, Y., et al., 2000. Stepwise assembly of a glucocorticoid receptor hsp90 heterocomplex resolves two sequential ATP-dependent events involving first hsp70 and then hsp90 in opening of the steroid binding pocket. J. Biol. Chem. 275 (24), 18054–18060.
Muck-Seler, D., et al., 1999. Platelet serotonin, plasma cortisol, and dexamethasone suppression test in schizophrenic patients. Biol. Psychiatry 45 (11), 1433–1439.
Muck-Seler, D., et al., 2004. Platelet serotonin and plasma prolactin and cortisol in healthy, depressed and schizophrenic women. Psychiatry Res. 127 (3), 217–226.
Nimgaonkar, V.L., et al., 2017. The complement system: a gateway to gene-environment interactions in schizophrenia pathogenesis. Mol. Psychiatry 22 (11), 1554–1561.
van Nimwegen, L.J., et al., 2008. Hepatic insulin resistance in antipsychotic naive schizophrenic patients: stable isotope studies of glucose metabolism. J. Clin. Endocrinol. Metab. 93 (2), 572–577.
Perkovic, M.N., et al., 2017. Theranostic biomarkers for schizophrenia. Int. J. Mol. Sci. 18 (4).
Perlman, W.R., et al., 2004. Reduced glucocorticoid and estrogen receptor alpha messenger ribonucleic acid levels in the amygdala of patients with major mental illness. Biol. Psychiatry 56 (11), 844–852.
Perlman, W.R., et al., 2007. Age-related differences in glucocorticoid receptor mRNA levels in the human brain. Neurobiol. Aging 28 (3), 447–458.
Phassouliotis, C., et al., 2013. Enhanced cortisol suppression following administration of low-dose dexamethasone in first-episode psychosis patients. Aust. N. Z. J. Psychiatry. 47 (4), 363–370.
Phillips, L.J., et al., 2006. Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. Aust. N. Z. J. Psychiatry. 40 (9), 725–741.
Rao, S., et al., 2016. Common variants in FKBP5 gene and major depressive disorder (MDD) susceptibility: a comprehensive meta-analysis. Sci. Rep. 6, 32687.
Raymond, C., et al., 2018. Early child adversity and psychopathology in adulthood: HPA axis and cognitive dysregulations as potential mechanisms. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 85, 152–160.
Ritsner, M., et al., 2004. Elevation of the cortisol/dehydroepiandrosterone ratio in schizophrenia patients. Eur. Neuropsychopharmacol. 14 (4), 267–273.
Sasse, S.K., et al., 2015. Response element composition governs correlations between binding site affinity and transcription in glucocorticoid receptor feed-forward loops. J. Biol. Chem. 290 (32), 19756–19769.
Scharf, S.H., et al., 2011. Expression and regulation of the Fkbp5 gene in the adult mouse brain. PLoS One 6 (2), e16883.
Schiene-Fischer, C., Yu, C., 2001. Receptor accessory folding helper enzymes: the functional role of peptidyl prolyl cis/trans isomerases. FEBS Lett. 495 (1–2), 1–6.
Sinclair, D., et al., 2011. Abnormal glucocorticoid receptor mRNA and protein isoform expression in the prefrontal cortex in psychiatric illness. Neuropsychopharmacology 36 (13), 2698–2709.
Sinclair, D., et al., 2013. Dysregulation of glucocorticoid receptor co-factors FKBP5, BAG1 and PTGES3 in prefrontal cortex in psychotic illness. Sci. Rep. 3, 3539.
Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch. Gen. Psychiatry 60 (12), 1187–1192.
Sullivan, P.F., Fan, C., Perou, C.M., 2006. Evaluating the comparability of gene expression in blood and brain. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141b (3), 261–268.
Szczepankiewicz, A., et al., 2014. FKBP5 polymorphism is associated with major depression but not with bipolar disorder. J. Affect. Disord. 164, 33–37.
Walker, E.F., Diforio, D., 1997. Schizophrenia: a neural diathesis-stress model. Psychol. Rev. 104 (4), 667–685.
Walker, E.F., et al., 2013. Cortisol levels and risk for psychosis: initial findings from the North American prodrome longitudinal study. Biol. Psychiatry 74 (6), 410–417.
Watson, S., et al., 2012. A randomized trial to examine the effect of mifepristone on neuropsychological performance and mood in patients with bipolar depression. Biol. Psychiatry 72 (11), 943–949.
Webster, M.J., et al., 2002. Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. Mol. Psychiatry 7 (9), 985–994, 924.
Wechsler, D., 1997. Wechsler Adult Intelligence Scale - Third Edition. The Psychological Corporation, San Antonio, Texas.
Wechsler, D., 2001. Wechsler Test of Adult Reading. The Psychological Corporation, San Antonio, Texas.
Weickert, C., et al., 2016. FKBP5 messenger RNA increases after adolescence in human dorsolateral prefrontal cortex. Biol. Psychiatry 80 (5), e29–e31.
Weickert, C.S., et al., 2013. Biomarkers in schizophrenia: a brief conceptual consideration. Dis. Markers 35 (1), 3–9.
Wochnik, G.M., et al., 2005. FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J. Biol. Chem. 280 (6), 4609–4616.
Yung, A.R., et al., 2004. Risk factors for psychosis in an ultra high-risk group: psychopathology and clinical features. Schizophr. Res. 67 (2–3), 131–142.