Psychiatry Research 280 (2019) 112510

Contents lists available at ScienceDirect

Psychiatry Research

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

Dissecting the syndrome of schizophrenia: Associations between symptomatology and hormone levels in women with schizophrenia

Natalie Thomas*, Caroline Gurvich, Abdul-Rahman Hudaib, Emorfia Gavrilidis, Jayashri Kulkarni

Monash Alfred Psychiatry Research Centre, Monash University Central Clinical School and The Alfred Hospital, Level 4, 607 St Kilda Rd Melbourne, Australia

ARTICLE INFO

Keywords:
Schizophrenia
Hormones
Heterogeneity
Group based trajectory modelling

ABSTRACT

Despite many studies implicating reproductive hormones in the development and outcome of schizophrenia, few have characterised the association between symptomatology and hormonal trajectories. To understand the influence of hormones on schizophrenia symptoms, serum steroids (estradiol, progesterone, follicular stimulating hormone (FSH), luteinising hormone (LH), and dehydroepiandrosterone (DHEA)) and psychopathology (The positive-and-negative-syndrome-scale(PANSS)) and depression (Montgomery-Asberg-Depression-Rating Scale (MADRS)) were collected across 12-weeks in 45 women (mean age 46) diagnosed with schizophrenia. To account for potential heterogeneity, Group-based-trajectory-modelling of psychopathology was used to identify distinct subgroups of individuals following a similar pattern of association between symptom score and hormone levels over-time. Two trajectories were identified for PANSS: one subgroup with lower symptom severity was associated with FSH, DHEA, LH, and another high severity subgroup associated with LH. Two trajectories were identified for MADRS: 'depressed' (associated with FSH), and non-depressed. The result delineates subpopulations with unique psychopathology and hormone associations that support the hypothesis that reproductive hormones play a role in the pathophysiology of schizophrenia, and that heterogeneity may exist in hormonal sensitivities in the schizophrenia population. Stratification of subjects according to biological phenotype may help improve existing treatments through personalised-medicine strategies. The endocrine system may be one such biological mechanism to continue dissecting the syndrome.

1. Introduction

Schizophrenia is a complex, psychiatric disorder with diverse behavioural and cognitive symptoms, which may reflect etiological heterogeneity (Takahashi, 2013). Although the pathophysiology of schizophrenia remains unclear, it is likely that several genetic and environmental factors interact to govern a multitude of neurotransmitter systems in the brain implicated in schizophrenia, including the dopaminergic, glutamatergic, gamma-aminobutyric acid (GABA), cholinergic, and serotonergic systems (Yang and Tsai, 2017). Several biological mechanisms have been proposed to explain, at least in part, the pathological modulation of such neurotransmission, including immunological, metabolic, and endocrine homeostatic systems (Guest et al., 2011; Landek-Salgado et al., 2016).

Increasing evidence from clinical, in vitro, and animal data suggests that the hypothalamic-pituitary-gonadal (HPG) endocrine axis, particularly estradiol, plays a pivotal role in the development and functioning of the brain (Galea et al., 2017; Gobinath et al., 2017; McEwen and Milner, 2017), and has been suggested to be implicated in the course and outcome for schizophrenia (Hafner, 2003). It has been hypothesised that estradiol is neuroprotective, supported by evidence of better outcomes and a later disease onset in females as compared to males. Menstrual cycle studies also report a worsening of schizophrenia symptoms during phases of low estradiol as well as a second prevalence peak in women during the postmenopausal period when estradiol levels decline (Gogos et al., 2015). In addition, women of reproductive age with diagnosed schizophrenia are often hypo-estrogenic, with frequent reports of menstrual irregularities (Huber et al., 2001). Notably, clinical data support the efficacy of adjunctive estradiol and selective estrogen receptor modulators (SERMs) in the treatment of schizophrenia, particularly for positive symptoms (Akhondzadeh et al., 2003; Kulkarni et al., 2016a). Although studies demonstrating a direct association between estradiol levels and schizophrenia psychopathology are sparse, and primarily cross-sectional in nature, a negative correlation between circulating estradiol levels and symptoms of schizophrenia has been described (Hoff et al., 2001), in addition to one study using regression analysis to demonstrate a significant inverse relationship between estradiol levels and change of schizophrenia symptoms across the

* Corresponding author.
E-mail address: natalie.thomas@monash.edu (N. Thomas).

https://doi.org/10.1016/j.psychres.2019.112510
Received 27 June 2019; Received in revised form 5 August 2019; Accepted 6 August 2019
Available online 08 August 2019
0165-1781/

menstrual cycle phase (Bergemann et al., 2007).

The literature linking reproductive hormones to schizophrenia is dominated by the 'estrogen neuroprotective hypothesis', although research considering the role of other gonadal hormones such as progesterone in schizophrenia also exists (Sun et al., 2016) Other fields, such as neurology, have recognised the potential contribution of a number of reproductive hormones to brain development, brain function and brain disease (for example Alzheimer's disease and multiple sclerosis) (Ysrraelit and Correale, 2019). Progesterone and progesterone metabolites (eg. allopregnanolone), luteinising hormone (LH), follicular stimulating hormone (FSH), testosterone and the precursor to estradiol and testosterone, dihydroepiandrosterone (DHEA) have all been implicated in brain and behaviour. The levels of sex steroid hormones are controlled by homeostatic feedback, with a complex interplay of hormones regulating itself and often regulating each other, which collectively influence mood and behaviour (Acevedo-Rodriguez et al., 2018).

It is increasingly accepted that biological abnormalities reported in schizophrenia have multiple causative pathways, with no single biological aetiology likely to be responsible for all cases. Supporting this notion, biological subgroups have been defined in schizophrenia based on immune dysregulation (presence of macrophage in brain tissue) (Cai et al., 2018b), plasma cytokine measures (Boerrigter et al., 2017), cortical surface complexities in frontal and temporal areas (Nenadic et al., 2014), and cholinergic muscarinic receptor density levels within the cortex (Scarr et al., 2009). Relevant to this paper, the identification of subgroups has also been demonstrated based on distinct differences in the molecular serum profiles of patients with schizophrenia, showing one group with predominant changes in immune molecules, and the other group with more significant changes in factors of the hormonal endocrine system (Schwarz et al., 2013).

Converging evidence indicates that heterogeneity is important at multiple levels of causation and that key pathways may be disrupted by combinations of many different genetic mutations (McClellan and King, 2010), including evidence for genetic factors contributing significantly to population variance in sex hormone levels (Ruth et al., 2016b). For example, there is considerable evidence that the single nucleotide polymorphism T allele of the FSH protein B (FSHB) promoter polymorphism decreases FSH levels (Ruth et al., 2016a; Schuring et al., 2013; Tuttelmann et al., 2012) and increased LH levels (Hayes et al., 2015; Ruth et al., 2016b) whilst haplotypes consisting of two polymorphisms (rs2234693 and rs9340799) in the estrogen receptor alpha (ER α) gene may be strongly associated with schizophrenia, particularly with regards to age of onset, general psychopathology and therapeutic effects (Wang et al., 2013).

Despite many studies implicating HPG hormones both in the development and outcome of schizophrenia, few studies have specifically investigated how gonadal steroid hormones and their precursor pituitary hormone levels directly associate with psychopathology, and a mechanistic understanding of the role reproductive hormones play in schizophrenia remains elusive. Studies to date have been limited and inconsistent, with majority reporting on estradiol levels only, and often using a cross-sectional study design to evaluate the relationship with symptomatology or predict diagnosis. As hormonal endocrine factors are known to fluctuate over time, it is important to adopt a longitudinal design. Additionally, research also suggests that hormonal sensitivity is not universal and instead may appear in subgroups of individuals who are 'differentially sensitive to the impact of reproductive steroids on the central nervous system' (Rubinow and Schmidt, 2018). Although majority of studies investigating hormone levels and schizophrenia assume disease homogeneity, it is important to recognise that biological subgroups may exist within the diagnostic umbrella of schizophrenia/schizoaffective disorder diagnoses. Therefore, to ascertain the temporal association between HPG hormone levels and schizophrenia psychopathology trajectory, serum sex steroids and pituitary hormones (estradiol, progesterone, FSH, LH and DHEA) and schizophrenia and depression psychopathology scores were collected over four time points (baseline, week 4, week 8, week 12). To account for potential heterogeneity, Group based trajectory modelling (GBTM) assessment of psychopathology growth trajectory, effected by time-variant hormone levels under a conditional model, was utilised.

2. Methods and study design

2.1. Study design

The data for the present study represents pooled longitudinal data of the placebo arm from two clinical trials in which the primary aim was to determine the effects of adjunctive raloxifene treatment on symptoms of psychosis in women with schizophrenia in child bearing aged women, and older women (Kulkarni et al., 2016b). Trials were conducted according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines (Moher et al., 2010) and conducted as parallel-design, 12-week, double-blind RCT. The original studies are registered at ClinicalTrials.gov Identifier: NCT00361543 and NCT02354001.

2.2. Participants

Women were eligible if they met criteria for schizophrenia or schizoaffective disorder and were receiving a stable dose of antipsychotics for at least 4 weeks before enrolment. They were required to be physically well, and excluded if currently taking any hormonal treatments (including contraceptives or hormonal replacement therapy (HRT)). The present analyses included forty-five participants who had been randomly assigned to the placebo arm, of which had at least two time points of recorded psychopathology and hormone levels. The Alfred Human Research Ethics Committee approved all study protocols, and all participants were provided with written informed consent after receiving a complete description of the study.

2.3. Measures and procedure

Participants' psychiatric and medical histories were recorded at screening, in addition to the Mini-International Neuropsychiatric Interview to confirm psychiatric diagnosis. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Depression was assessed using the Montgomery–Asberg Depression Rating Scale (MADRS), a 10-item researcher rated scale of depression (Montgomery and Asberg, 1979). Relevant clinical factors for the current study were age, duration of illness (DOI), body mass index (BMI), menstrual cycle status, and anti-psychotic use. Females were classified as having regular or irregular cycles based on their menstrual cycle histories, as per previously reported methodology (Gleeson et al., 2015). Details of menstrual cycle regularity, the date of the last menstrual cycle, and usual menstrual cycle length were asked of participants and menses were classified as regular if they occurred once every 3–5 weeks. Cycle lengths outside this time frame were considered irregular and included women with amenorrhoea and women in menopause. A single 10 mL blood sample was collected at 4 time points to assay for serum sex steroids and pituitary hormone levels (including estradiol, progesterone, LH and FSH, and dehydroepiandrosterone (DHEA)). Serum levels were assayed at the Alfred Pathology service via electrochemiluminescence method (ECLIA) or chemiluminescent microparticles immunoassay (CMIA).

3. Statistical analysis

The effects of serum hormones were measured from baseline to week 12 with total PANSS and MADRS score progression (outcomes), using group-based trajectory models (GBTM). This semi-parametric technique is used to identify distinct subgroups of individuals following

a similar pattern of association of change over time on a given variable (Nagin, 2005). Assuming patterns of associations between symptoms and hormones are heterogenous within the studied population, variation reflects underlying differences in such associations and their strength. Given each subject has a unique developmental trajectory, the heterogeneity or the distribution of individual differences within the data is summarised by a finite set of unique linear functions each corresponding to a discrete subgroup (Nagin, 2005). In this paper, we fitted conditional GBTM models to simultaneously characterise outcome trajectories with time-variant covariates (*tcov* = hormone levels) and test the presence of distinct or meaningful subgroups that were identified with dimensions of time and repeated hormone levels. Each subgroup or subpopulation consisted of subjects of similar latent trajectories. The selection of the best fitting solution with the optimal subgroup number or trajectory order was based on the Bayesian information criteria (BIC) (Jones and Nagin, 2007), and the theoretical basis of the produced subgroups.

Subsequently, the posterior probabilities of subgroup memberships were estimated. To ensure that specific hormone-conditioned PANSS or MADRS trajectory patterns were not influenced or explained by sample observed variates or factors like age, schizophrenia duration of illness (DOI), antipsychotic medication dose (defined as risperidone equivalents), BMI and the menstruating status, sample variables were used to predict the trajectory subgroups using logistic regression or cross tabulation (Fisher's exact test) and the odd ratios across subgroups were reported. Non-log-transformed values (raw scores) for hormone levels were used in the tested models due to the mean and variance for each variable data being minimally correlated (Shanmugam, 2008). Further, the multivariate distributions for time-variant variables conformed to Gaussian density estimates. All models were written with, Proc Traj (Jones and Nagin, 2007) (SAS institute, Cary NC).

4. Results

4.1. Sample variables

Participants included 45 female patients with mean age of 46.5(SD = 11.8), duration of illness of chronic schizophrenia of 20.4 years (SD = 11.2), mean BMI of 31.7(SD = 7.6) and mean dose of antipsychotic medications of 6.1 mg (SD = 4.5, in risperidone-equivalents). Menstruation strata variable was binary with 49% (n = 22) of participants identified as non-menstruating. Information of menstrual cycle phase at baseline were collected (4 women were follicular, 5 were luteal phase, 14 menstruating, but unknown phase at baseline.) There were no significant differences or changes between participants with any of the sample variables from baseline to the end of follow up.

4.2. Growth curve models (GCM)

We first conducted a one-group model or GCM in which the intercept, linear, quadratic slopes (if fit) means and variances were freely estimated. The variance around the mean PANSS (best fit: intercept-only, M = 74.8 SE = 1.9) trajectory were **166.7**, $p < 0.0001$ (Fig. 1a). For MADRS trajectory (best fit: linear, $M = -1.3$, $SE = 0.4$, $d = -0.85$), the intercept variance was **64.3**, $p = 0.0004$ (Fig. 2a). The significant variability in each trajectory and the marginal fit indices (CFI, TLI < 0.95 for both models), indicated sufficient heterogeneity and further statistically justified the use of GBTM.

4.3. PANSS trajectory subgroups

A *priori* knowledge was not known for the number of subgroups for each model, therefore, based on BIC indices for trajectories, parsimony and theoretical value, a two- subgroup solution (for each conditional model) was deemed as best fit (very strong evidence, (Jones et al., 2001), (Tabachnick & Fidell, 2007). The final models are displayed in Figs. (1, 2) and fit indices in Table 1 Supplementary Material.

For FSH and DHEA conditioned- PANSS trajectories (models), the first subgroup, labelled "low severity", included over half of participants (53.2%, 56.0%, respectively) whom stable PANSS subgroup (intercept-only) was negatively predicted by FSH ($b = -0.15, p < 0.001$) (Fig. 1b) and positively associated with DHEA ($b = 1.45$, $p < 0.001$) (Fig. 1c). For both models, the first subgroup, labelled 'high severity', characterised with higher stable PANSS scores, was not predicted by FSH or DHEA. For LH model, the conditioned two- PANSS trajectories were both significantly predicted by LH (Fig. 1d). However, evident by a larger regression weight, participants in the second subgroup (58.8%), showed a stronger negative association with LH ($b = -0.37$, $p < 0.001$). Estradiol and progesterone time scores effect on PANSS response were not significantly patterned or fit with any tested order (intercept-only to quadratic polynomial, all $ps > 0.10$).

4.4. MADRS trajectory subgroups

Best-fitting models were selected based on BIC criteria. The two-subgroup conditioned- MADRS trajectories for FSH, LH, DHEA, estradiol and progesterone models revealed the second subgroup, labelled "depressed descending" represented varying proportions or posterior probabilities of subgroup membership by each model (53.9%, 50.4%, 46.3%, 49.2%, 42.2%, respectively) and displayed higher MADRS scores ('depressed'), and were characterised by significant negative regression mean estimates for linear slopes ($b = -1.69$, $b = -1.50$, $b = -1.80$, $b = -1.93$, $b = -1.89$, respectively). However, only the pattern of MADRS linear trajectory response in the second subgroup was significantly predicted by FSH ($b = -0.07$, $p = 0.03$) and not by the other hormone variates (Fig. 2b).

4.5. Predicting trajectory subgroups

After fitting both PANSS and MADRS two- subgroup models, predictors (sample variables, Section 4.1) were used as covariates to estimate the likelihood of a participant's membership in subgroup 2 ('low severity') compared to subgroup 1 with logistic regression or cross tabulation. Results are reported in Table 1. Briefly, the odd ratios and their 95% confidence intervals, showed no statistically significant predictors across the two subgroups, for both PANSS and MADRS models. Thus, sample characteristics or predictors, while relevant (in theory), did not explain or account for participant's varying trajectories for PANSS or MADRS conditioned models.

5. Discussion

The design presented delineates a subpopulation with unique psychopathology and hormone association trajectories. The 'low severity' subgroup, characterised by stable low PANSS scores, demonstrated as association with FSH, LH, and DHEA steroid levels, whilst 'high severity' schizophrenia' PANSS score trajectory was predicted by LH only, albeit at a lesser effect size than the other subgroup. The 'depressed' subpopulation characterised by descending MADRS score over time demonstrated a conditional effect of FSH over time, in contrast to no association demonstrated in the other 'non-depressed' subgroup. These collective results support the broad hypothesis that sex hormones play a role in the pathophysiology of schizophrenia, and suggest that heterogeneity may exist with regards to hormonal sensitivities in this female schizophrenia population. Such heterogeneity agrees with the growing consensus that different biological phenomena may underlie differential subgroups within the syndrome. Why the association between symptoms (PANSS) and hormone levels was observed principally in the 'low severity' schizophrenia subgroup remains unclear, but may reflect a patient subgroup in which the underlying pathophysiology of hormone modulation results in less severe schizophrenia symptomatology. An alternative hypothesis may be that symptoms of greater

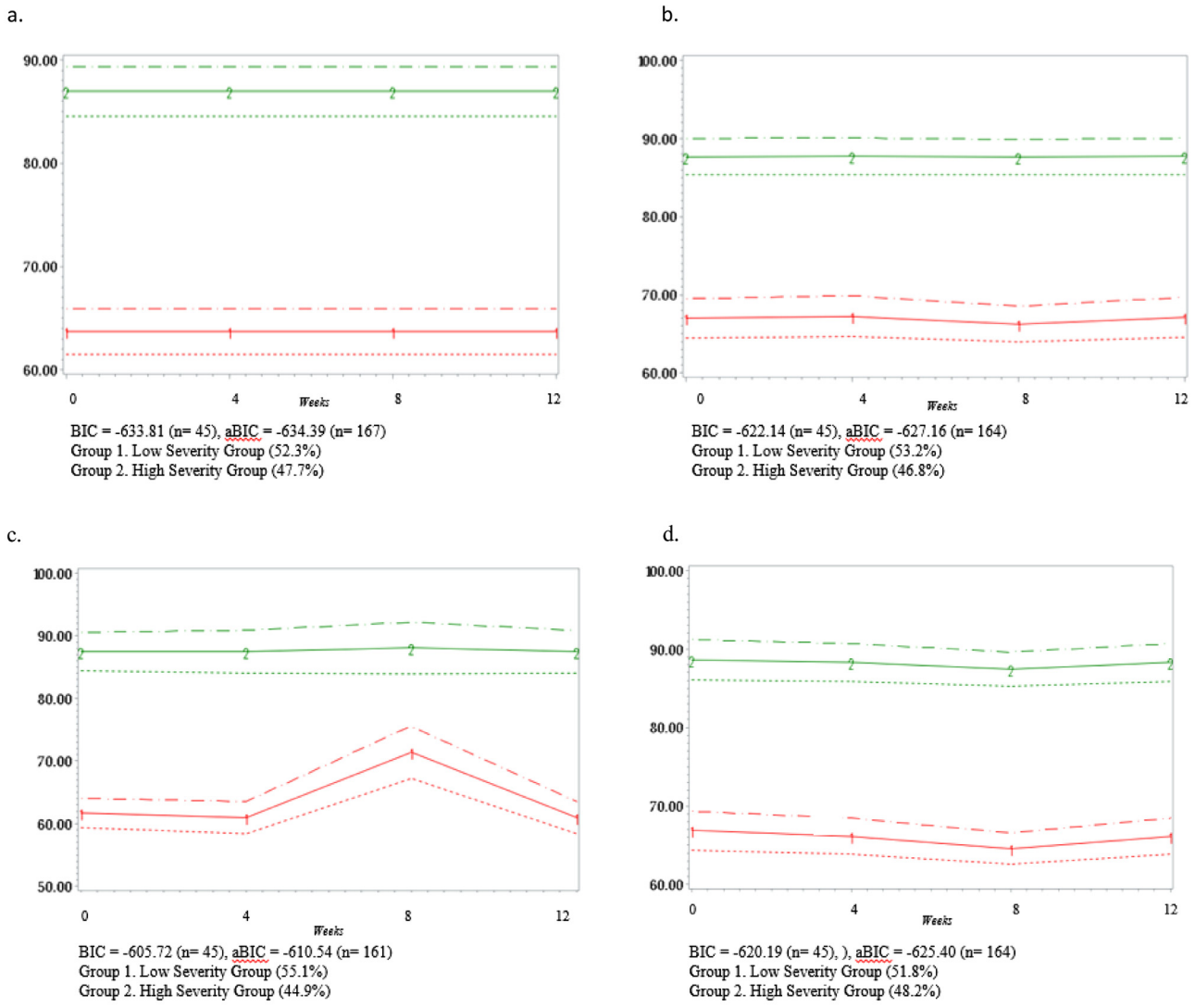

Fig. 1. Group-based trajectory groups for PANSS (a.) Unconditional model (b.) FSH conditional model group 1 ($B0 = -0.15$, SE = 0.04, $p < 0.001$), group 2 ((B0 = −0.016, $SE = 0.02$, $p = 0.39$), (c.) DHEA conditional model group1 ($B0 = 1.45$, SE = 0.39, $p < 0.001$), group2 ($B0 = 0.07$, $SE = 0.30$, $p = 0.81$), (d.) LH conditional model group1 ($B0 = -0.37$, SE = 0.1, $p < 0.001$), group2 (($B0 = -0.18$, $SE = 0.08$, $p = 0.03$). Lines (numbered) represent each group trajectory or curve across time (x axis), Y axis represents symptom outcome scores, $B0$ represents intercept regression coefficient.

schizophrenia severity cannot be modulated by hormonal levels. Future characterisation of subtypes based on outcome or severity and associative biological markers is warranted.

A positive correlation between DHEA and PANSS symptom trajectory is largely consistent with the literature. A recent meta-analysis demonstrated a pooled schizophrenia patient cohort had elevated levels of DHEA-S (the sulphated version of DHEA) compared to non-psychiatric, although significance remained only for first-episode schizophrenia male patients subsequent to sensitivity (subgroup) analysis (Misiak et al., 2018). Moreover, a study reported after the published meta-analysis showed elevated serum levels of DHEA-S in post-menopausal women with diagnosed schizophrenia compared to matched controls, and a positive correlation observed between severity levels defined by SANS scores and DHEA-S levels (Bulut et al., 2018).

Interestingly, a 2015 quantitative review of adjunctive DHEA treatment in schizophrenia showed no overall significant benefit in the active group (Heringa et al., 2015).

DHEA/DHEA-S, in addition to genomic neurosteroid action, has been shown to have fast-acting, non-genomic modulatory effects on neuronal excitability and synaptic plasticity, primarily working via positive allosteric modulation of the $GABA_A$ receptor (Vuksan-Cusa et al., 2016), which has been implicated in the pathophysiology of schizophrenia (Cai et al., 2018a). A relationship between DHEA and cortisol has also been described in the literature, proposing a DHEA anti-glucocorticoid antagonistic effect, resulting in protection against hippocampal neuronal cell death (Karishma and Herbert, 2002). It has therefore been suggested that upregulation of DHEA may be a compensatory mechanism in response to acute stress, in order to regulate cortisol activity (Belvederi Murri et al., 2012).

Limited research has been conducted exploring the relationship between pituitary hormones and schizophrenia symptomatology and psychiatric conditions generally. One cross-sectional study demonstrated a significant association between the ratio of FSH/LH and improvement in positive psychotic symptoms, but worsening of Clinical global Impression scale (CGI) total, and cognitive scores. This significance remained after adjusting for confounding variables but lost after Bonferroni correction for multiple testing. Individual FSH or LH levels were not significantly associated (Gonzalez-Rodriguez et al., 2017). The FSH/LH predicts response to controlled ovarian stimulation and is a useful biomarker in reproductive medicine predicting *in vitro* fertilisation outcome (Karishma and Herbert, 2002; Prasad et al., 2013). The authors suggest that either higher FSH and/or lower LH levels

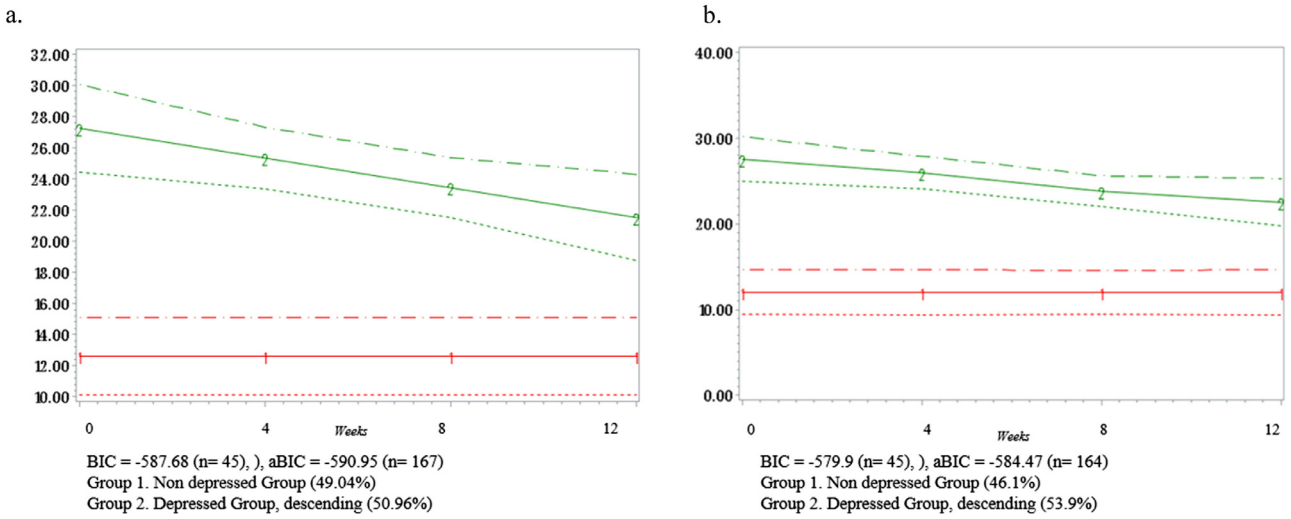

Fig. 2. Group-based trajectory groups for MADRS (a.) Unconditional model (b.) FSH conditional model group 1 ($B0 = 0.0004$, $SE = 0.02$, $p = 0.98$), group 2 ($B1 = -0.07$, $SE = 0.03$, $p = 0.03$). Lines (numbered) represent each group trajectory or curve across time (x axis), Y axis represents symptom outcome scores, $B0$ represents intercept regression coefficient.

Table 1
Odd ratios* of sample information predictors to conditional PANSS/MADRS trajectories.

PANSS trajectory model conditioned on time and FSH	
Sample predictor($N = 45$)	Group 1 (low severity)†
Age, in years	1.04 [0.98–1.09]
Body mass index	1.00 [0.92–1.08]
Illness duration, in years	0.98 [0.93–1.04]
Antipsychotic dose (risperidone-equivalent)	0.97 [0.85–1.11]
Non-menstruating/menstruating	3.11 [0.72–14.83]
PANSS trajectory model conditioned on time and LH	
Sample predictor	Group 1 (low severity)†
Age, in years	1.03 [0.98–1.09]
Body mass index	1.00 [0.92–1.07]
Illness duration, in years	0.98 [0.93–1.03]
Antipsychotic dose (risperidone-equivalent)	0.92 [0.79–1.06]
Non- menstruating/menstruating	2.50 [0.59–11.34]
PANSS trajectory model conditioned on time and DHEA	
Sample predictor	Group 1 (low severity)†
Age, in years	1.04 [0.98–1.10]
Body mass index	0.99 [0.91–1.07]
Illness duration, in years	1.00 [0.94–1.05]
Antipsychotic dose (risperidone-equivalent)	0.96 [0.84–1.10]
Non-menstruating/menstruating	3.96 [0.89–20.37]
MADRS trajectory model conditioned on time and FSH	
Sample predictor	Group 2 (Depressed Descending)ǂ
Age, in years	0.97 [0.92–1.02]
Body mass index	1.04 [0.96–1.13]
Illness duration, in years	1.02 [0.96–1.07]
Antipsychotic dose (risperidone-equivalent)	0.97 [0.85–1.11]
Non-menstruating/menstruating	1.69 [0.40–7.47]

* All $ps > 0.05$.
† Reference group was group 2 (high severity).
ǂ Reference group was group 1(non-depressed), []: 95% confidence intervals.

could potentially predict improvement in positive symptoms (Gonzalez-Rodriguez et al., 2017). Higher FSH agrees with the current data demonstrating a negative association between FSH and total PANSS trajectory, in the 'low severity' subgroup. As FSH hormone levels have been implicated in both depressive mood symptoms and suicidality (Kim et al., 2013; Ryan et al., 2009), and schizophrenia psychotic symptoms (Gonzalez-Rodriguez et al., 2017), the current results demonstrating an influence of steroidal FSH on both PANSS and MADRS scores is consistent with a growing literature.

Interestingly, despite a growing body of double blind, randomised, placebo controlled trials demonstrating that adjunctive estradiol treatment is efficacious for schizophrenia symptoms, providing compelling evidence for the 'estrogen protection hypothesis' (Riecher-Rössler et al., 2018), neither estradiol nor progesterone showed an effect on either outcome (PANSS, MADRS) regardless of subgroup. This suggests that although exogenous estradiol treatment may influence the endocrine system culminating in beneficial effect, this may result in an indirect, upstream effect at the level of the pituitary hormones, or that peripheral pituitary hormones may provide a better measure of CNS activity than gonadal estradiol levels in a patient group not hormonally treated

Pituitary hormones LH, FSH are regulated by both the negative and positive feedback of estradiol depending on cycle stage. Prior to ovulation within the menstrual cycle, estradiol switches from a negative to a positive feedback effect, initiating the surge of pituitary hormone levels that produces ovulation (Kurbel, 2012). Although this complex mechanism is not yet completely understood, at moderate, constant levels of circulating estradiol, a negative feedback effect of LH secretion occurs, whilst during the cycle, an elevated estrogen level exerts a predominant positive feedback effect and stimulates LH secretion (Barrett and Ganong, 2012). It is therefore conceivable that increased endogenous estradiol levels via estradiol supplementation may exert a positive feedback upregulation of pituitary hormones that may modulate symptomatology, in the direction suggested by the presented data. Nevertheless, the molecular mechanism underlying FSH and LH action in schizophrenia pathophysiology remains unknown, and future work to elucidate this potential mechanism is required.

Assessment of potential predictors DOI, BMI, age, menstrual cycle status, and medication use could not fully explain the differential subgroup trajectories conditioned on hormonal levels, suggesting that an unobserved variable is responsible for the two biological subgroups, with differential relationships with temporal hormone levels. Therefore, in this cohort, we hypothesise that a subpopulation of 'low severity schizophrenia' and the 'depressed group' may have intrinsic difference in their response to pituitary and gonadal hormones.

6. Strengths and limitations

Although this paper has several strengths, including the longitudinal design, the modelling of multiple hormones levels as time variant independent covariates, and the modern statistical methods utilised to

explore hormones conditioned clusters or subgroups for both outcomes, findings should be interpreted in light of a number of limitations. Firstly, the sample size may have limited the power to test models with higher subgroup numbers, however, the subgroups produced by GBTM are not immutable and are model based (Nagin, 2005). Moreover, trajectories in different patient and treatment groups remain to be evaluated. Secondly, variables, other than the fitted predictors, may have better explained participant's subgroup membership, however, predictors in our models are widely and conceptually relevant to PANSS and MADRSS outcomes and endocrine regulation in the literature. In addition, GBTM subgroups (Jones and Nagin, 2007) are hypothesised to be accounted for by unobserved or partially observed variates. Further, the measurement of peripheral hormone levels was used in an effort to approximate central bioavailability and assumed central and peripheral levels of steroids were correlated. Although sex hormones can easily traverse the blood-brain-barrier due to their high lipid solubility, it should be acknowledged that peripheral levels of steroids may not reflect levels in the central nervous system (CNS), due to the regional variability in neural tissue (Heringa et al., 2015), and the intrinsic ability of the brain to synthesis local neurosteroids. However, positive correlations have been demonstrated in rat between plasma levels and brain levels of neurosteroids (Barbaccia et al., 2001), and in humans between plasma and cerebral spinal fluid (CSF) levels (Kim et al., 2000).

Despite these limitations, this study is the first, to our knowledge, to characterise and cluster the conditional effects of pituitary and sex hormones on psychopathology scores in a sample of female patients with schizophrenia.

Conclusions

Stratification of patients according to biological phenotype may help to improve existing treatments through personalised medicine strategies. The endocrine system may be one such biological mechanism to continue dissecting the syndrome of schizophrenia.

Declaration of Competing Interest

Dr. Natalie Thomas reports no biomedical financial interests or potential conflicts of interest

Dr. Caroline Gurvich reports no biomedical financial interests or potential conflicts of interest

Dr. Abdul Hudaib reports no biomedical financial interests or potential conflicts of interest

Ms. Emorfia Gavrilidis reports no biomedical financial interests or potential conflicts of interest

Professor Jayashri Kulkarni reports no biomedical financial interests or potential conflicts of interest

Acknowledgements

Sources of funding; Grant support

• Grant 546084 National Health and Medical Research Council Project Grant 2009, 2009–2011. Brain Estrogens—A Potential Treatment for Symptoms of Schizophrenia?

• Grant 1007762 National Health and Medical Research Council Project Grant 2011, 2011–2013. Selective estrogen receptor modulators: a potential new treatment for women of child-bearing age with psychotic symptoms of schizophrenia

Original studies registered at ClinicalTrials.gov Identifier: NCT00361543 and NCT02354001.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.112510.

References

Acevedo-Rodriguez, A., Kauffman, A.S., Cherrington, B.D., Borges, C.S., Roepke, T.A., Laconi, M., 2018. Emerging insights into hypothalamic-pituitary-gonadal axis regulation and interaction with stress signalling. J Neuroendocrinol. 30 (10), e12590.

Akhondzadeh, S., Nejatisafa, A.A., Amini, H., Mohammadi, M.R., Larijani, B., Kashani, L., Raisi, F., Kamalipour, A., 2003. Adjunctive estrogen treatment in women with chronic schizophrenia: a double-blind, randomized, and placebo-controlled trial. Prog. Neuropsychopharmacol. Biol. Psychiatry 27 (6), 1007–1012.

Barbaccia, M.L., Affricano, D., Purdy, R.H., Maciocco, E., Spiga, F., Biggio, G., 2001. Clozapine, but not haloperidol, increases brain concentrations of neuroactive steroids in the rat. Neuropsychopharmacology 25 (4), 489–497.

Barrett, K.E., Ganong, W.F., 2012. Ganong's Review of Medical Physiology. McGraw-Hill Medical, New York.

Belvederi Murri, M., Pariante, C.M., Dazzan, P., Hepgul, N., Papadopoulos, A.S., Zunszain, P., Di Forti, M., Murray, R.M., Mondelli, V., 2012. Hypothalamic-pituitary-adrenal axis and clinical symptoms in first-episode psychosis. Psychoneuroendocrinology 37 (5), 629–644.

Bergemann, N., Parzer, P., Runnebaum, B., Resch, F., Mundt, C., 2007. Estrogen, menstrual cycle phases, and psychopathology in women suffering from schizophrenia. Psychol. Med. 37 (10), 1427–1436.

Boerrigter, D., Weickert, T.W., Lenroot, R., O'Donnell, M., Galletly, C., Liu, D., Burgess, M., Cadiz, R., Jacomb, I., Catts, V.S., Fillman, S.G., Weickert, C.S., 2017. Using blood cytokine measures to define high inflammatory biotype of schizophrenia and schizoaffective disorder. J. Neuroinflammation 14 (1), 188.

Bulut, S.D., Bulut, S., Gundogmus, A.G., Aydemir, C., 2018. Serum DHEA-S, testosterone and cortisol levels in female patients with schizophrenia. Endocr. Metab. Immune Disord. Drug Targets 18 (4), 348–354.

Cai, H., Cao, T., Zhou, X., Yao, J.K., 2018a. Neurosteroids in schizophrenia: pathogenic and therapeutic implications. Front. Psychiatry 9, 73.

Cai, H.Q., Catts, V.S., Webster, M.J., Galletly, C., Liu, D., O'Donnell, M., Weickert, T.W., Weickert, C.S., 2018b. Increased macrophages and changed brain endothelial cell gene expression in the frontal cortex of people with schizophrenia displaying inflammation. Mol. Psychiatry.

Galea, L.A.M., Frick, K.M., Hampson, E., Sohrabji, F., Choleris, E., 2017. Why estrogens matter for behavior and brain health. Neurosci. Biobehav. Rev. 76 (Pt B), 363–379.

Gleeson, P.C., Worsley, R., Gavrilidis, E., Nathoo, S., Ng, E., Lee, S., Kulkarni, J., 2015. Menstrual cycle characteristics in women with persistent schizophrenia. Aust. N. Z. J. Psychiatry 50 (5), 481–487.

Gobinath, A.R., Choleris, E., Galea, L.A., 2017. Sex, hormones, and genotype interact to influence psychiatric disease, treatment, and behavioral research. J. Neurosci. Res. 95 (1–2), 50–64.

Gogos, A., Sbisa, A.M., Sun, J., Gibbons, A., Udawela, M., Dean, B., 2015. A role for estrogen in schizophrenia: clinical and preclinical findings. Int. J. Endocrinol. 2015 615356-615356.

Gonzalez-Rodriguez, A., Bernardo, M., Penades, R., Arias, B., Ruiz Cortes, V., Seeman, M.V., Catalan, R., 2017. Do FSH/LH ratio and gonadal hormone levels predict clinical improvement in postmenopausal schizophrenia women? Arch. Womens Ment. Health 20 (5), 613–620.

Guest, P.C., Martins-de-Souza, D., Vanattou-Saifoudine, N., Harris, L.W., Bahn, S., 2011. Abnormalities in metabolism and hypothalamic-pituitary-adrenal axis function in schizophrenia. Int. Rev. Neurobiol 101, 145–168.

Hafner, H., 2003. Gender differences in schizophrenia. Psychoneuroendocrinology 28 (Suppl 2), 17–54.

Hayes, M.G., Urbanek, M., Ehrmann, D.A., Armstrong, L.L., Lee, J.Y., Sisk, R., Karaderi, T., Barber, T.M., McCarthy, M.I., Franks, S., Lindgren, C.M., Welt, C.K., Diamanti-Kandarakis, E., Panidis, D., Goodarzi, M.O., Azziz, R., Zhang, Y., James, R.G., Olivier, M., Kissebah, A.H., Stener-Victorin, E., Legro, R.S., Dunaif, A., 2015. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. Nat. Commun. 6, 7502.

Heringa, S.M., Begemann, M.J., Goverde, A.J., Sommer, I.E., 2015. Sex hormones and oxytocin augmentation strategies in schizophrenia: a quantitative review. Schizophr. Res. 168 (3), 603–613.

Hoff, A.L., Kremen, W.S., Wieneke, M.H., Lauriello, J., Blankfeld, H.M., Faustman, W.O., Csernansky, J.G., Nordahl, T.E., 2001. Association of estrogen levels with neuropsychological performance in women with schizophrenia. Am. J. Psychiatry 158 (7), 1134–1139.

Huber, T.J., Rollnik, J., Wilhelms, J., von zur Muhlen, A., Emrich, H.M., Schneider, U., 2001. Estradiol levels in psychotic disorders. Psychoneuroendocrinology 26 (1), 27–35.

Jones, B.L., Nagin, D.S., Roeder, K., 2001. A SAS procedure based on mixture models for estimating developmental trajectories. Sociol. Methods Res. 29, 374–393.

Jones, B.L., Nagin, D.S., 2007. Advances in group-based trajectory modeling and an SAS procedure for estimating them. Sociol. Methods Res. 35 (4), 542–571.

Karishma, K.K., Herbert, J., 2002. Dehydroepiandrosterone (DHEA) stimulates neurogenesis in the hippocampus of the rat, promotes survival of newly formed neurons and prevents corticosterone-induced suppression. Eur. J. Neurosci. 16 (3), 445–453.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 13 (2), 261–276.

Kim, B., Kang, E.S., Fava, M., Mischoulon, D., Soskin, D., Yu, B.H., Lee, D., Lee, D.Y., Park, H.D., Jeon, H.J., 2013. Follicle-stimulating hormone (FSH), current suicidal ideation and attempt in female patients with major depressive disorder. Psychiatry Res. 210 (3), 951–956.

Kim, Y.S., Zhang, H., Kim, H.Y., 2000. Profiling neurosteroids in cerebrospinal fluids and plasma by gas chromatography/electron capture negative chemical ionization mass

spectrometry. Anal. Biochem. 277 (2), 187–195.
Kulkarni, J., Gavrilidis, E., Gwini, S.M., Worsley, R., Grigg, J., Warren, A., Gurvich, C., Gilbert, H., Berk, M., Davis, S.R., 2016a. Effect of adjunctive raloxifene therapy on severity of refractory schizophrenia in women: a randomized clinical trial. JAMA Psychiatry 73 (9), 947–954.
Kulkarni, J., Gavrilidis, E., Gwini, S.M., Worsley, R., Grigg, J., Warren, A., Gurvich, C., Gilbert, H., Berk, M., Davis, S.R., 2016b. Effect of adjunctive raloxifene therapy on severity of refractory schizophrenia in women: a randomized clinical trialeffect of adjunctive raloxifene therapy in refractory schizophreniaeffect of adjunctive raloxifene therapy in refractory schizophrenia. JAMA Psychiatry 73 (9), 947–954.
Kurbel, S., 2012. A phase plane graph based model of the ovulatory cycle lacking the "positive feedback" phenomenon. Theor. Biol. Med. Model. 9 35–35.
Landek-Salgado, M.A., Faust, T.E., Sawa, A., 2016. Molecular substrates of schizophrenia: homeostatic signaling to connectivity. Mol. Psychiatry 21 (1), 10–28.
McClellan, J., King, M.-.C., 2010. Genetic heterogeneity in human disease. Cell 141 (2), 210–217.
McEwen, B.S., Milner, T.A., 2017. Understanding the broad influence of sex hormones and sex differences in the brain. J. Neurosci. Res. 95 (1–2), 24–39.
Misiak, B., Frydecka, D., Loska, O., Moustafa, A.A., Samochowiec, J., Kasznia, J., Stanczykiewicz, B., 2018. Testosterone, DHEA and DHEA-S in patients with schizophrenia: a systematic review and meta-analysis. Psychoneuroendocrinology 89, 92–102.
Moher, D., Hopewell, S., Schulz, K.F., Montori, V., Gotzsche, P.C., Devereaux, P.J., Elbourne, D., Egger, M., Altman, D.G., 2010. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. J. Clin. Epidemiol. 63 (8), e1–37.
Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 382–389.
Nagin, D., 2005. Group-based modeling of development. Cambridge: Harvard University, Press.
Nenadic, I., Yotter, R.A., Sauer, H., Gaser, C., 2014. Cortical surface complexity in frontal and temporal areas varies across subgroups of schizophrenia. Hum. Brain Mapp. 35 (4), 1691–1699.
Prasad, S., Gupta, T., Divya, A., 2013. Correlation of the day 3 FSH/LH ratio and LH concentration in predicting IVF outcome. J. Reprod. Infertil. 14 (1), 23–28.
Riecher-Rössler, A., Butler, S., Kulkarni, J., 2018. Sex and gender differences in schizophrenic psychoses—a critical review. Arch Womens Ment. Health 21 (6), 627–648.
Rubinow, D.R., Schmidt, P.J., 2018. Is there a role for reproductive steroids in the etiology and treatment of affective disorders? Dialogues Clin. Neurosci. 20 (3), 187–196.
Ruth, K.S., Beaumont, R.N., Tyrrell, J., Jones, S.E., Tuke, M.A., Yaghootkar, H., Wood, A.R., Freathy, R.M., Weedon, M.N., Frayling, T.M., Murray, A., 2016a. Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause and impact female reproductive health. Hum. Reprod. 31 (2), 473–481.
Ruth, K.S., Campbell, P.J., Chew, S., Lim, E.M., Hadlow, N., Stuckey, B.G.A., Brown, S.J., Feenstra, B., Joseph, J., Surdulescu, G.L., Zheng, H.F., Richards, J.B., Murray, A., Spector, T.D., Wilson, S.G., Perry, J.R.B., 2016b. Genome-wide association study with 1000 genomes imputation identifies signals for nine sex hormone-related phenotypes. Eur. J. Hum. Genet. 24 (2), 284–290.
Ryan, J., Burger, H.G., Szoeke, C., Lehert, P., Ancelin, M.L., Henderson, V.W., Dennerstein, L., 2009. A prospective study of the association between endogenous hormones and depressive symptoms in postmenopausal women. Menopause 16 (3), 509–517.
Scarr, E., Cowie, T.F., Kanellakis, S., Sundram, S., Pantelis, C., Dean, B., 2009. Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. Mol. Psychiatry 14 (11), 1017–1023.
Schuring, A.N., Busch, A.S., Bogdanova, N., Gromoll, J., Tuttelmann, F., 2013. Effects of the FSH-beta-subunit promoter polymorphism -211G->T on the hypothalamic-pituitary-ovarian axis in normally cycling women indicate a gender-specific regulation of gonadotropin secretion. J. Clin. Endocrinol. Metab. 98 (1), E82–E86.
Schwarz, E., van Beveren, N.J.M., Ramsey, J., Leweke, F.M., Rothermundt, M., Bogerts, B., Steiner, J., Guest, P.C., Bahn, S., 2013. Identification of subgroups of schizophrenia patients with changes in either immune or growth factor and hormonal pathways. Schizophr. Bull. 40 (4), 787–795.
Shanmugam, R., 2008. Correlation between the sample mean and sample variance. J. Mod. Appl. Stat. Methods 7 (2), 6.
Sun, J., Walker, A.J., Dean, B., van den Buuse, M., Gogos, A., 2016. Progesterone: the neglected hormone in schizophrenia? A focus on progesterone-dopamine interactions. Psychoneuroendocrinology 74, 126–140.
Tabachnick, B.G., Fidell, L.S., 2007. Using Multivariate Statistics (5th ed.). New York, Allyn and Bacon.
Takahashi, S., 2013. Heterogeneity of schizophrenia: genetic and symptomatic factors. Am. J. Med. Genet. B Neuropsychiatr. Genet. 162b (7), 648–652.
Tuttelmann, F., Laan, M., Grigorova, M., Punab, M., Sober, S., Gromoll, J., 2012. Combined effects of the variants FSHB -211G>T and FSHR 2039A>G on male reproductive parameters. J. Clin. Endocrinol. Metab. 97 (10), 3639–3647.
Vuksan-Cusa, B., Sagud, M., Rados, I., 2016. The role of dehydroepiandrosterone (DHEA) in schizophrenia. Psychiatr. Danub. 28 (1), 30–33.
Wang, S., Li, W., Zhao, J., Zhang, H., Yang, Y., Wang, X., Yang, G., Lv, L., 2013. Association of estrogen receptor alpha gene polymorphism with age at onset, general psychopathology symptoms, and therapeutic effect of schizophrenia. Behav. Brain Funct. 9 12–12.
Yang, A.C., Tsai, S.-.J., 2017. New targets for schizophrenia treatment beyond the dopamine hypothesis. Int. J. Mol. Sci. 18 (8), 1689.
Ysrraelit, M.C., Correale, J., 2019. Impact of sex hormones on immune function and multiple sclerosis development. Immunology 156 (1), 9–22.