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Research Article

RESEARCH ARTICLE

Reduced mRNA Expression of *PTGDS* in Peripheral Blood Mononuclear Cells of Rapid-Cycling Bipolar Disorder Patients Compared with Healthy Control Subjects

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Abstract

Background: Disturbances related to the arachidonic acid cascade and prostaglandin metabolism may be involved in the pathophysiology of bipolar disorder, as supported by a recent genome-wide association study meta-analysis; however, evidence from clinical studies on a transcriptional level is lacking. Two enzymes in the arachidonic acid cascade are the prostaglandin D synthase (PTGDS), which catalyzes the conversion of prostaglandin H2 to prostaglandin D2 (PGD_2), and the aldo-keto reductase family 1 member C3 (AKR1C3), which catalyzes the reduction of PGD_2. We aimed to test the hypothesis that mRNA expression of *PTGDS* and *AKR1C3* is deregulated in rapid-cycling disorder patients in a euthymic or current affective state compared with healthy control subjects, and that expression alters with affective states.
Methods: *PTGDS* and *AKR1C3* mRNA expression in peripheral blood mononuclear cells was measured in 37 rapid-cycling bipolar disorder patients and 40 age- and gender-matched healthy control subjects using reverse transcription quantitative real-time polymerase chain reaction. Repeated measurements of *PTGDS* and *AKR1C3* mRNA expression were obtained in various affective states during 6–12 months and compared with repeated measurements in healthy control subjects.
Results: Adjusted for age and gender, *PTGDS* mRNA expression was down-regulated in rapid-cycling bipolar disorder patients in a euthymic, depressive, and manic/hypomanic state compared with healthy control subjects. No difference in *PTGDS* mRNA expression was observed between affective states. *AKR1C3* mRNA expression did not differ between bipolar disorder patients in any affective state or in comparison with healthy control subjects.
Conclusions: The results suggest a role for aberrantly-regulated *PTGDS* mRNA expression in rapid-cycling bipolar disorder. The sample size was limited; replication of the findings in larger, independent samples is warranted to further explore the role of the arachidonic acid cascade and prostaglandin metabolism as a potential therapeutic target in bipolar disorder.

Keywords: *AKR1C3*,.biomarker, bipolar disorder, *PTGDS*, rapid cycling

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Introduction

Disturbances within the arachidonic acid (AA) cascade have been proposed as a pathophysiological mechanism in the regulation of mood and suggested to contribute to the underlying biological background for bipolar disorder (Bazinet, 2010). This is corroborated by preclinical evidence pointing to the arachidonic cascade as a target of drugs used to manage bipolar disorder (Rapoport and Bosetti, 2002; Bazinet, 2009), indicating that lithium (Galimberti et al., 2014), valproate (Kieseppa et al., 2014), carbamazepine (Lee et al., 2012), and antipsychotics (Cheon et al., 2011) decrease AA turnover in the brain. In contrast, the antidepressants imipramine and fluoxetine, which may induce mania, increase brain AA turnover, while bupropion, which may be at lower risk of inducing mania (Post et al., 2006), does not alter AA turnover (Lee et al., 2007, 2010). These findings have led to the formulation of the arachidonic acid theory of bipolar disorder (Bazinet, 2009). Additional evidence of downstream disturbances in the cascade, with increased levels of AA-derived prostaglandins in saliva (Ohishi et al., 1988), cerebrospinal fluid (Linnoila et al., 1983), and peripheral blood (Lieb et al., 1983; Calabrese et al., 1986) of mood disorder patients, have supported a role for this pathway in bipolar disorder. In a recent genome-wide association study meta-analysis, one of three novel loci identified to be associated with bipolar disorder was near the PTGFR gene encoding the prostaglandin F receptor (Chen et al., 2013), which is highly expressed in the brain.

Arachidonic acid, particularly abundant in the brain, is a polyunsaturated fatty acid present in the cell membrane phospholipids, from which it is freed by cytosolic phospholipase A2 (cPLA$_2$; Rapoport, 2014). The isoenzyme cPLA$_2$ IVA is selective for AA hydrolysis and its activity is modulated by lithium and carbamazepine (Rapoport et al., 2009; Berg et al., 2010). The expression of cPLA$_2$ IVA, as well as of the cyclooxygenase (COX)-2 enzyme, has been found to be altered in the post-mortem brain tissue of bipolar disorder patients (Kim et al., 2011). AA acts as a precursor in the production of prostaglandin H2 (PGH$_2$), mediated by COX, which, in turn, is converted to prostaglandins, thromboxanes, or prostacyclins (Funk, 2001). The conversion of PGH$_2$ to prostaglandin D2 (PGD$_2$) is catalyzed by prostaglandin D synthase (PTGDS), encoded by the *PTGDS* gene, and preferentially expressed in the brain. PGD$_2$ functions as a neuromodulator as well as a trophic factor in the central nervous system (Taniguchi et al., 2007) and is preferentially expressed in the brain. Reduction of both PGH$_2$ and PGD$_2$ is catalyzed by the aldo-keto reductase family 1 member C3 (AKR1C3) enzyme, encoded by the *AKR1C3* gene, resulting in synthesis of prostaglandin F2 alpha (Figure 1).

Evidence of AA cascade and prostaglandin pathway dysregulation on a transcriptional level in bipolar disorder is limited. Two studies by the same group, a case study (n = 1; Begemann et al., 2008) and an extended case series (n = 4; Gurvich et al., 2014) of rapid-cycling bipolar disorder patients identified the *PTGDS* and *AKR1C3* as differentially regulated between manic and depressive episodes and a case-control study of children and young adults with bipolar disorder (n = 9) and ADHD (Marín-Méndez et al., 2012) found the *PTGDS* gene was differentially expressed between bipolar disorder patients and patients with ADHD.

In bipolar disorder, which is characterized by phenotypically distinct, recurrent episodes of various polarities, gene expression alterations have the potential to inform on pathophysiological processes related to illness activity and affective state and to the nature of the illness itself. Surprisingly, beyond the two case studies (Begemann et al., 2008; Gurvich et al., 2014), gene expression changes longitudinally between affective states have not been investigated, and no studies have included assessment of healthy control subjects of these specific genes. In a recent meta-analysis of 17 studies of gene expression alterations in peripheral blood in bipolar disorder patients, comprising 565 patients and 418 healthy control subjects (Munkholm et al., 2012), we showed that findings were limited overall by lack of replication across studies and limited control for possible confounders of gene expression levels.

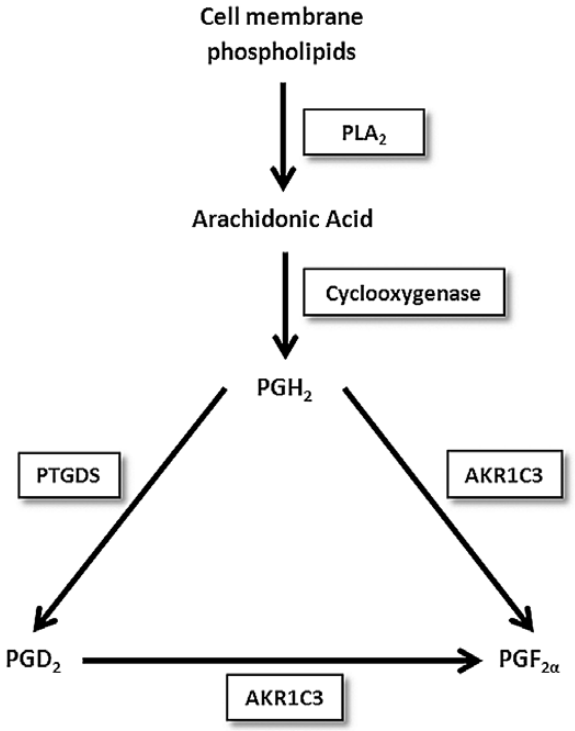

Figure 1. The arachidonic acid cascade and prostaglandin metabolism pathway related to the function of PTGDS and AKR1C3. AKR1C3, aldo-keto reductase family 1 member C3; PGD$_2$, prostaglandin D2; PGF$_{2\alpha}$, prostaglandin F2α; PGH$_2$, prostaglandin H2; PLA$_2$, phospholipase A2; PTGDS, prostaglandin D synthase.

The present study is the first to investigate repeated measures over time of the gene expression of *PTGDS* and *AKR1C3* in rapid-cycling bipolar disorder patients in a euthymic or current affective state and in healthy control subjects.

We hypothesized that mRNA expression of *PTGDS* and *AKR1C3* was deregulated in patients in a euthymic or current affective state compared with healthy control subjects as well as in bipolar disorder patients between current affective states (depressed, manic/hypomanic, or mixed) compared with those in the euthymic state. A longitudinal, naturalistic design was employed, accommodating assessment of patients during multiple affective states of varying polarity.

Methods and Materials

Participants

Bipolar Disorder Patients

Patients with a potential diagnosis of rapid-cycling bipolar disorder were recruited through referral by psychiatrists at hospitals or outpatient facilities throughout the region of Zealand, Denmark, with study recruitment taking place during the period of June 2010 to May 2012. Inclusion criteria were: adults aged 18–70 years; and a *Diagnostic and Statistical Manual of Mental Disorders*,

4th version (DSM-IV) diagnosis of rapid-cycling bipolar disorder, defined by the occurrence of at least four mood episodes (mania, hypomania, depression, or mixed) during the preceding year in the context of bipolar disorder. Exclusion criteria were: significant physical illness (ie chronic heart disease, chronic pulmonary disease, inflammatory disease, chronic infectious disease, or neurodegenerative disease); current drug abuse; insufficient Danish language skills; and pregnancy. A total of 37 bipolar disorder patients were included. Two bipolar patients declined further examination after one and three month follow-ups, respectively; the remaining bipolar patients were followed for a minimum of six months with a mean (standard deviation [SD]) follow-up period of 11.9 (3.0) months. Upon signs of new affective episodes, patients were evaluated with clinical assessments of mood and collection of blood samples which, when possible, were repeated after return to a subsequent euthymic state or change to an affective episode of opposite polarity. In cases of clinical signs of acute infection, any allergic symptoms, or any other acute medical condition, assessment and biochemical analysis were postponed. Blood samples were, on average, collected from bipolar patients 3.4±1.7 (range, 1–10) times during the study. Samples were obtained during euthymia in 34 patients (mean 2.0±1.3 [0–6]), major depression in 26 patients (mean 1.7±1.7 [0–5]), mania/hypomania in 11 patients (mean 0.7±1.2 [0–5]), and in a mixed state in a total of 6 patients (mean 0.2±0.4 [0–1]).

Healthy Control Subjects

Forty healthy control subjects were recruited among blood donors affiliated with the Blood Bank at Rigshospitalet, Copenhagen. Inclusion criteria were: adults aged 18–70 years; and no history of psychiatric disorder in the subjects or their first-degree relatives. Exclusion criteria were identical to those applied to bipolar disorder patients. Healthy control subjects were evaluated with clinical assessments and collection of blood samples on two separate occasions approximately three months apart. Assessment and biochemical analysis were postponed if there were clinical signs of acute infection, any allergic symptoms, or any other acute medical condition. Mean (SD) follow-up time for the healthy control subjects was 2.9 (0.9) months.

All participants provided written informed consent and were reimbursed for their travel expenses. The study protocol was approved by the Committee on Health Research Ethics of the Capital Region of Denmark (protocol no. H-4-2010-006). The study complied with the Declaration of Helsinki.

Clinical Assessments

All participants were assessed by a specialist in psychiatry (Dr Munkholm), using standardized semi-structured interviews. The Schedules for Clinical Assessment in Neuropsychiatry interview (Wing et al., 1990) was used for diagnostic purposes and was based on available case material, referral reports, the interview with the participant, and the Hypomania Checklist (Angst et al., 2005), completed by the participant. A DSM-IV diagnosis of rapid-cycling bipolar disorder was established for the patients and comorbid psychiatric illness, if present, was recorded. For healthy control subjects, absence of lifetime psychiatric morbidity was confirmed.

A clinical diagnosis according to DSM-IV, without applying duration criteria, was established at each study visit concurrently with the collection of samples for laboratory analysis. Severity of depressive symptoms was assessed using the 17-item Hamilton Depression Rating Scale (HAMD-17; Hamilton, 1967), employing a structured interview guide (Williams, 1990) translated to Danish, and manic symptoms were assessed using the Young Mania Rating Scale (YMRS; Young et al., 1978), with a time period of three days applied. Medication, alcohol intake, and smoking habits during the two weeks prior to assessment were recorded.

Categories of affective states were based on clinical evaluation according to the Schedules for Clinical Assessment in Neuropsychiatry interview combined with the HAMD-17 and YMRS rating scales without applying duration criteria: euthymic (HAMD-17 and YMRS < 8), depressive (HAMD-17 > 7 and YMRS < 8), manic/hypomanic (YMRS > 7 and HAMD-17 < 8), and mixed state (HAMD-17 > 7 and YMRS > 7).

Blood Draw and Peripheral Blood Mononuclear Cell Isolation

Blood samples were obtained in the fasting state between 0830 and 1030 hours, after a minimum period of 15 minutes rest, concurrently with the clinical evaluation.

Nine milliliters of blood were drawn by venipuncture into a citrate phosphate dextrose adenine–containing vacuum tube (Vacuette), which was kept at room temperature before and after the blood draw.

Peripheral blood mononuclear cells (PBMC) were collected applying the standard Ficoll-Paque PLUS isolation procedure (GE Healthcare Life Sciences), within one hour of blood draw. PBMC were aliquoted into 1.5 ml Eppendorf tubes and kept frozen at -80°C until assayed.

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from PBMC by use of TRIzol reagent (Life Technologies). RNA quality and quantification was measured spectrophotometrically using a NanoDrop (NanoDrop Technologies) spectrophotometer and software, applying the 260/280 and 260/230 ratio algorithms. cDNA was synthesized from RNA with a High Capacity dDNA Reverse Transcription Kit (Life Technologies). The cDNA was subjected to quantitative real-time polymerase chain reaction (PCR) using the ViiA 7 Real-Time PCR System (Life Technologies) with SYBR Green PCR Master Mix (Life Technologies). Gene-specific sequence oligonucleotide primers (*PTGDS*, *AKR1C3*, *GAPDH*, *TBP*, and *SDHA*) were purchased from TAG Copenhagen.

A set of three genes, the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene, the TATA box binding protein (*TBP*) gene, and the succinate dehydrogenase complex, subunit A, flavoprotein (*SDHA*) gene, were used as candidate reference genes for normalization. The sequences of all primers used are presented in Table 1.

The stability of candidate reference genes was assessed using the NormFinder software (Akhondzadeh et al., 2009). *SDHA* exhibited the highest stability in comparisons between bipolar disorder patients and healthy control subjects, as well as between affective states in bipolar disorder patients (*SDHA* = 0.139; *TBP* = 0,322; *GAPDH* = 0.271), and no combination of two genes showed higher stability. Semi-quantitative *PTGDS* and *AKR1C3* mRNA levels, assessed by cycle threshold (C_T) were thus expressed relative to *SDHA*. We measured the ΔC_T = C_T (each gene) - C_T (*SDHA*) for each sample. Relative levels of expression were determined using the comparative C_T method (Ricken et al., 2013) method, calculated by $2^{-\Delta C_T}$. All assays were performed in triplicate with laboratory personnel blinded to the clinical status of participants.

In addition, standard clinical chemistry parameters were analyzed, including fasting blood glucose and fasting lipid parameters.

Statistics

Independent t-tests were used to test differences in age between healthy control subjects and bipolar disorder patients, and the chi-squared test was used to examine differences in categorical demographic and clinical variables.

For our main analyses we employed a two-level linear mixed effects model, accommodating both variation of the outcome variables within subjects (intra-individual variation) and between subjects (inter-individual variation). Level one represented repeated measures of *PTGDS* and *AKR1C3* mRNA levels and level two represented between-subject variation. We conducted two separate sets of analyses, one on comparisons between bipolar disorder patients and healthy control subjects (set A) and one on comparisons between affective states among bipolar disorder patients (set B). In both sets, unadjusted mixed-model analyses with expression levels of each gene as the dependent variables were firstly conducted (model A-1 and B-1) followed by several *a priori* models specified within each set of analyses (A and B, models 2–4).

All models included a random intercept to accommodate correlations in the outcome variables over time within each participant. All other covariates were specified as fixed effects. The assumptions of independence of errors, homoscedasticity, and normality were met.

To evaluate the correlation between levels of *PTGDS* and *AKR1C3* expression, a Pearson's correlation analysis was performed, using residual values produced by our mixed model (model A-2).

The statistical analysis was conducted with SPSS, version 22.0 (IBM Corporation).

Results

Demographic and Clinical Characteristics

There were no significant differences between bipolar disorder patients and healthy control subjects with regard to age, gender, educational level, or BMI. More patients were smokers but alcohol consumption was higher among healthy control subjects (Table 2).

All of the study participants were Caucasian. Bipolar disorder patients were overall on stable medication for a month before study entry and during the course of the study, with few participants changing medication despite alterations of affective state,

Table 1. Primer Sequences.

Gene	Forward primer (5' to 3')	Backward primer (3' to 5')
PTGDS	'CGGCTCCTACAGCTACCG'	'CAGCGCGTACTGGTCGTA'
AKR1C3	'CATTGGGGTGTCAAACTTCA'	'CCGGTTGAAATACGGATGAC'
GAPDH	'CTGACTTCAACAGCGACACC'	'TGCTGTAGCCAAATTCGTTGT'
TBP	'GAACATCATGGATCAGAACAACAG'	'ATAGGGATTCCGGGAGTCAT'
SDHA	'GAGGCAGGGTTTAATACAGCAT'	'CCAGTTGTCCTCCTCCATGTT'

AKR1C3, aldo-keto reductase family 1 member C3; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *PTGDS*, prostaglandin D synthase; *SDHA*, succinate dehydrogenase complex, subunit A, flavoprotein; *TBP*, TATA box binding protein.

Table 2. Demographic and Clinical Characteristics of the Study Participants at Inclusion.

	Bipolar Disorder Patients	Healthy control subjects	Statistic	*p*-value
N	37	40		
Age (years)	40.9 ± 12.3 (23–66)	36.3 ± 12.5	$t = 1.828$	0.1
Gender (female-male)	25-12	23-17	$Chi^2 = 0.830$	0.3
Education (years total)	16.1 ± 3.0 (9–22)	16.4 ± 2.3 (10–21)	$t = 0.608$	0.5
Number of smokers (%)	21 (56.8)	1 (2.5)	$Chi^2 = 18.547$	<0.0001
Alcohol consumption (units per week)	1.7 ± 2.4 (0–10)	5.1 ± 4.4 (0–15)	$t = 4.120$	<0.0001
Alcohol intake > 14 units/week (%)	0 (0)	2 (5.0)	$Chi^2 = 1.899$	0.168
Body Mass Index	24.6 ± 3.6 (19.4–32.4)	24.9 ± 3.9 (20.0–42.2)	$t = 0.353$	0.7
Duration of illness (years)	21.2 ± 13.0 (2–56)			
Bipolar I (%)	22 (59.5)			
Bipolar II (%)	15 (40.5)			
Number of depressive episodes	16.2 ± 15.4 (2–60)			
Number of hypomanic episodes	16.5 ± 19.1 (2–92)			
Number of manic episodes	3.2 ± 7.1 (0–35)			
Number of hospitalizations	10.2 ± 19.5 (0–75)			
Lithium treatment (%)	15 (40.5)			
Anticonvulsant treatment (%)	27 (73.0)			
Antipsychotic treatment (%)	27 (73.0)			
SSRI treatment (%)	8 (21.6)			
Newer antidepressant treatment (%)	2 (5.4)			
Older antidepressant treatment (%)	2 (5.4)			

Data are expressed as mean ± standard deviation (range) or n (%). SSRI, selective serotonergic reuptake inhibitors.

owing to the fact that the majority of patients received intensive outpatient treatment and, despite pharmacologically advanced treatment, continually experience affective episodes. Four patients stopped and one patient started selective serotonin reuptake inhibitor treatment, two patients started lithium treatment, two started anticonvulsant treatment, and one started antipsychotic treatment during the study period. One bipolar disorder patient suffered from co-morbid obsessive-compulsive disorder; no participants suffered from comorbid generalized anxiety disorder. Four bipolar disorder patients reported mild, well-controlled hypertension and two reported mild, intermittent reflux esophagitis. One healthy control subject reported intermittent symptoms of allergic rhinitis, but not during the study period.

The majority of the patients received specialized treatment at the Mood Disorders Clinic, Psychiatric Center Copenhagen, Rigshospitalet, Copenhagen, Denmark, and all of the patients were outpatients at the time of inclusion.

Symptom severity of participants at the time of assessment and sampling are presented in Table 3.

PTGDS and *AKR1C3* mRNA Expression in Bipolar Disorder Patients Compared with Healthy Control Subjects

In an unadjusted analysis (model A-1), lower levels of *PTGDS* mRNA expression were observed in all affective states compared with healthy control subjects; however, only between a euthymic state and healthy control subjects was this difference statistically significant (b = -0.060, 95% confidence interval [CI; -0.117; -0.002], p = 0.041). No difference was observed in an unadjusted analysis for *AKR1C3* mRNA expression between bipolar disorder patients in any affective state and healthy control subjects [F(4, 184.86) = 0.343, p = 0.8]. Adjusting for age and gender (model A-2), a statistically significant down-regulation of *PTGDS* mRNA expression was present in bipolar disorder patients in both the euthymic state (b = -0.073, 95% CI [-0.130; -0.017], p = 0.012), the depressive state (b = -0.062, 95% CI [-0.120; -0.003], p = 0.038), and the manic/hypomanic state (b = -0.076, 95% CI [-0.144; -0.008], p = 0.028), while the lower levels observed in a mixed state did not reach statistical significance (b = -0.053, 95% CI [-0.143; 0.037], p = 0.2; Figure 2A). In an adjusted analysis (model A-2), no difference was observed in *AKR1C3* mRNA expression between bipolar disorder patients in any affective state compared with healthy control subjects [F(4, 184.66) = 0.207, p = 0.9; Figure 2B]. Age, but not gender, was weakly but positively associated with both *PTGDS* mRNA expression (b = 0.003, 95% CI [0.001; 0.005], p = 0.007) and *AKR1C3* mRNA expression (b = 0.001, 95% CI [0.000; 0.001], p = 0.01). In an exploratory analysis of clinical and demographical variables (BMI and alcohol intake) possibly associated with *PTGDS* and *AKR1C3* mRNA expression (model A-3), where smokers were excluded because of uneven distribution between groups, alcohol intake was not associated with *PTGDS* mRNA expression (p = 0.2) or *AKR1C3* mRNA expression (p = 0.5) and BMI was not associated with either *PTGDS* mRNA expression (p = 0.8) or *AKR1C3* expression (p = 0.2). In this exploratory analysis, only among bipolar patients in a manic/hypomanic state

Table 3. Symptom Severity of Participants at Time of Assessment.

	Samples from healthy control subjects	Samples from bipolar disorder patients n = 168			
		Euthymic	Depressive	Manic/hypomanic*	Mixed state
	n = 80	n = 75	n = 63	n = 24	n = 6
HAMD-17	0.6 ± 0.9 (0–3)	3.7 ± 1.9 (0–7)	15.5 ± 5.1 (8–27)	3.4 ± 2.6 (0–7)	10.2 ± 1.8 (8–12)
YMRS	0.4 ± 0.8 (0–3)	1.0 ± 1.7 (0–7)	0.9 ± 1.4 (0–6)	15.3 ± 4.3 (9–24)	11.2 ± 2.8 (8–16)

Data are expressed as mean ± standard deviation (range). N represents number of samples. Values are presented as raw values, unadjusted for repeated measures. HAMD-17, Hamilton rating scale, 17 items; YMRS, Young mania rating scale. *Manic patients, n = 19/Hypomanic patients, n = 5.

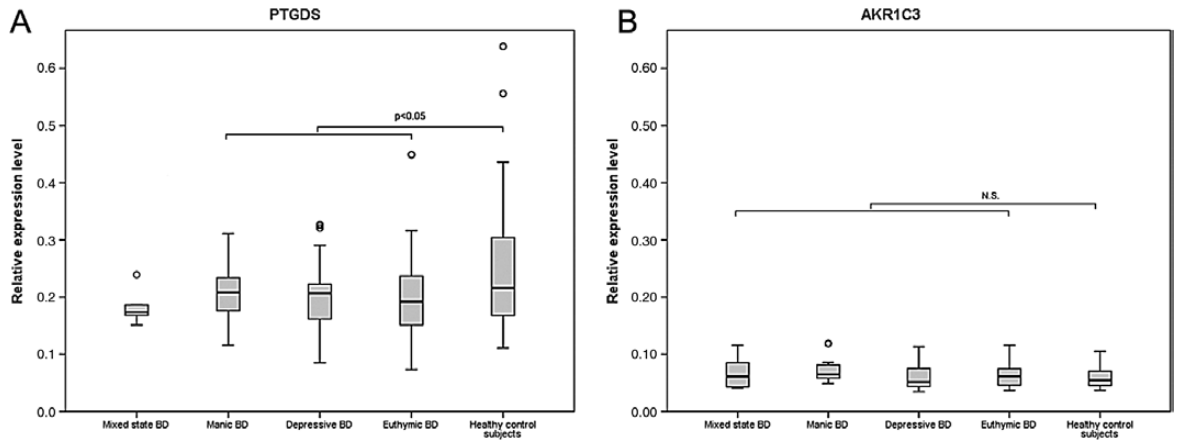

Figure 2. *PTGDS* (A) and *AKR1C3* (B) mRNA expression in rapid-cycling bipolar disorder patients and healthy control subjects. Levels represent back-transformed ΔC_T values based on a linear mixed-model analysis adjusted for age and gender (model A-2). *PTGDS* mRNA expression was down-regulated in rapid-cycling bipolar disorder patients in a euthymic (p = 0.01), depressive (p = 0.04), and manic/hypomanic (p = 0.03) state compared with healthy control subjects; no difference in *PTGDS* mRNA expression was observed between affective states. *AKR1C3* mRNA expression did not differ between affective states or between bipolar disorder patients and healthy control subjects. AKR1C3, aldo-keto reductase family 1 member C3; BD, bipolar disorder; C_T, cycle threshold; NS, not significant; PTGDS, prostaglandin D synthase.

was the down-regulated *PTGDS* mRNA expression compared with healthy control subjects statistically significant (b = -0.100, 95% CI [-0.0.197; -0.003], p = 0.044) with the non-significant findings for *AKR1C3* unaltered.

In a subgroup analysis including only bipolar disorder patients in a euthymic state of more than one month (model A-4), thus minimizing a possible effect of the previous episode on gene expression in a euthymic state, *PTGDS* mRNA expression remained significantly down-regulated in comparison to healthy control subjects (b = -0.073, 95% CI [-0.145; -0.001], p = 0.048), while there was no difference between groups for *AKR1C3* (p = 0.6).

State-Specific Alterations of *PTGDS* and *AKR1C3* mRNA Expression in Rapid-Cycling Bipolar Disorder Patients

In an unadjusted analysis (model B-1), there was no overall difference in mRNA expression between any current affective state and the euthymic state of either *PTGDS* [F(3, 139.820) = 0.208, p = 0.9] or *AKR1C3* [F(3, 138.188) = 0.294, p = 0.8]. There was also no difference in either *PTGDS* mRNA expression (p = 0.6) or *AKR1C3* mRNA expression (p = 0.4) between the depressed and the manic/hypomanic state. Adjusting for age and gender (model B-2) and subsequently adding the covariates BMI, smoking status (no/yes), alcohol intake, illness duration (≥10 years/<10 years) and medication (no/yes) in an exploratory analysis (model B-3) did not alter the results. In this analysis, none of the covariates entered into the model, including individual medications, were significantly associated with *PTGDS* or *AKR1C3* mRNA expression levels. Specifically, there was no effect of either lithium (b = -0.014, 95% CI [-0.067; 0.039], p = 0.6), anticonvulsant (b = -0.034, 95% CI [-0.091; 0.023], p = 0.2), antipsychotic (b = -0.001, 95% CI [-0.075; 0.075], p = 0.9), or antidepressant use (b = -0.005, 95% CI [-0.065; 0.056], p = 0.9) on *PTGDS* mRNA levels. Non-smoking status compared with smoking status was similarly not associated with PTGDS mRNA levels (b = 0.065, 95% CI [-0.016; 0.147], p = 0.1).

There was no association between HAMD-17 scores or YMRS scores and mRNA expression levels of *PTGDS* and *AKR1C3* in depressive and manic/hypomanic bipolar disorder patients, respectively (model B-4).

Post hoc subgroup analysis of patients not taking acetylsalicylic acid (n = 35) or statins (n = 33) during the study period revealed no difference in either *PTGDS* or *AKR1C3* mRNA expression between affective states (data not shown).

PTGDS and *AKR1C3* mRNA expression levels were weakly correlated [r(239) = 0.299, $p < 0.001$].

Discussion

This study investigated alterations of mRNA expression of *PTGDS* and *AKR1C3* between bipolar disorder and healthy control subjects and is the first study in a larger cohort to explore possible state-related alterations in expression of *PTGDS* and *AKR1C3*. We did this by employing a longitudinal design that allowed for assessment of gene expression in various affective states and incorporated both intra-individual and inter-individual alterations in mixed model analyses. In accordance with our hypothesis, *PTGDS* mRNA expression was altered in rapid-cycling bipolar disorder patients in euthymic, depressed, and manic/hypomanic states compared with healthy control subjects, with *PTGDS* expression down-regulated in patients compared with healthy control subjects. No difference in *AKR1C3* mRNA expression between patients and healthy control subjects was observed. Contrary to our hypothesis, mRNA expression of both *PTGDS* and *AKR1C3* did not differ between affective states in bipolar disorder patients.

The finding of down-regulated levels of *PTGDS* mRNA expression in peripheral blood in the current study is supported by findings in post-mortem brain tissue where both genes have been found down-regulated in frontal brain regions in bipolar disorder patients compared with healthy control subjects (Stanley Medical Research Institute Online Genomics Database). It would be of interest to elucidate possible single nuclear polymorphisms related to the *PTGDS* locus, which could further lead to mapping of potential expression quantitative trait loci. This analysis could prove especially useful in investigating differentially-regulated genes of interest between affective states in bipolar disorder.

Our results of comparable expression levels of *PTGDS* and *AKR1C3* across affective states in bipolar disorder patients are in contrast to the findings in two studies by the same group involving a total of five rapid-cycling disorder patients (Begemann et al., 2008; Gurvich et al., 2014), where *PTGDS* and *AKR1C3* expression were found to be down-regulated in depressive episodes compared with manic episodes. Our study consisted of a larger cohort of rapid-cycling disorder patients (n = 37), likely constituting a more representative sample of rapid-cycling bipolar disorder patients, involved a more rigorous methodological approach, such as transparent statistical analyses adjusted for relevant covariates, and used several reference genes that were tested for stability. Our cohort also represents a heterogeneous population of patients, and it is possible that subgroups of patients could exhibit a different pattern of gene expression. In the study finding *PTGDS* down-regulated in ADHD patients compared with bipolar disorder patients (Marín-Méndez et al., 2012), patients were both adolescents and adults and there was no information about the affective state or medication of participants.

Our finding of down-regulation of *PTGDS* could possibly represent a compensatory mechanism in reaction to an activated AA cascade in rapid-cycling bipolar disorder. Specifically, the down-regulation of *PTGDS* activity could represent a counter reaction to up-regulated PLA_2 or COX activity, as increases in PLA_2 activity in *in vivo* (Noponen et al., 1993) and of PLA_2 and COX-2 mRNA and protein levels in post-mortem bipolar brain tissue (Rao, Bazinet, et al., 2007) have been demonstrated. This is in line with findings from a recent study of bipolar disorder patient post-mortem brain tissue, where decreased expression of COX-1 and cytosolic prostaglandin E synthase were speculated to be compensatory to the increased expression of COX-2 and membrane prostaglandin E synthase also demonstrated in the study (Kim et al., 2011). The relatively weak correlation observed between *PTGDS* and *AKR1C3* mRNA expression levels may indicate that the role of the PTGDS and AKR1C3 enzymes in the AA cascade are not closely interrelated or that they are possibly differentially influenced by medication on a transcriptional level.

The finding of down-regulated *PTGDS* expression in rapid-cycling bipolar disorder patients across all affective states is also consistent with preclinical evidence suggesting down-regulation of the AA cascade as a mechanism of action for these medications. The majority of patients in our study were treated with lithium, anticonvulsants, or antipsychotics that, in preclinical studies, have been demonstrated to down-regulate the AA cascade (Bazinet, 2009; Rapoport et al., 2009), and it is possible that prolonged treatment with these medications not only results in normalizing an up-regulated AA cascade in these patients but even leads to down-regulation of the cascade below

normal activity. While there are no studies of the effect of mood-stabilizing medications on the mRNA expression of *PTGDS* and *AKR1C3* specifically, medication may also down-regulate mRNA expression of these genes. It is possible that under such circumstances, state-related alterations in *PTGDS* and *AKR1C3* mRNA expression do not occur. In the present study we did not find an effect of individual medication groups on either *PTGDS* or *AKR1C3* mRNA expression levels. However, the majority of the patients (78.4%) were treated with two or more medication groups and it is therefore difficult to assess the impact of individual medications. Along those lines, it cannot be excluded that treatment with multiple medication groups adds to the possible down-regulating effect of these medications on mRNA expression of *PTGDS* and *AKR1C3*. Investigation of larger cohorts may be necessary to elucidate the effect of individual medications on *PTGDS* and *AKR1C3* mRNA expression and of the AA cascade regulation in general. Such an effect is not only suggested by post-mortem brain findings but also by *in vivo* preclinical studies demonstrating that antipsychotics down-regulate AA metabolism (Cheon et al., 2011; Modi et al., 2013).

The present finding of aberrations in the AA cascade is in line with the current hypothesis on the pathophysiological background of bipolar disorder involving disturbances within several inter-related pathways, such as inflammatory system dysregulation (Goldstein et al., 2009), oxidative and nitrosative stress pathways (Maes et al., 2011), impairments in neuroplasticity (Duman and Monteggia, 2006), and mitochondrial dysfunction (Clay et al., 2011). Specifically, up-regulation of PLA_2 and COX-2 activity is observed upon activation of the inflammatory response system (Bauer et al., 1997; Adibhatla and Hatcher, 2007), which has been demonstrated to be activated in bipolar disorder (Munkholm et al., 2013), and elevated expression of PLA_2 and increased AA turnover have been found in animal models of neuroinflammation (Rao, Ertley, et al., 2007). Activation of the AA cascade can, conversely, also induce inflammatory responses and the production of pro-inflammatory cytokines (Munoz and Costa, 2013). Accumulation of AA generates intracellular reactive oxygen species (Magder, 2006), which can generate a state of oxidative stress that has been suggested to contribute to systemic toxicity (Kapczinski et al., 2010) and neuroprogression in bipolar disorder. Further, possible neuroprotective effects of mood-stabilizing treatment may be mediated through inhibition of the AA cascade (Rapoport et al., 2009) and AA cascade dysregulation may be involved in the pathophysiology underlying the neuroprogressive changes suggested in bipolar disorder (Berk, 2009) with excess AA possibly inducing apoptosis by damaging mitochondria (Saitoh et al., 2003). The mechanisms through which these pathways interact with disturbances in the AA cascade and relate to the clinically distinct affective states that constitute bipolar disorder, however, is unclear.

Genes expressed in the brain are to a large extent also expressed in peripheral blood, indicating that peripheral blood potentially could serve as a surrogate tissue (Liew et al., 2006; Le-Niculescu et al., 2009). The peripheral blood transcriptome may thus reflect system-wide biology, and it has further been demonstrated that a significant amount of single nuclear polymorphism expression relationships are conserved between the brain and peripheral blood lymphocytes (Iwamoto et al., 2011). It is unclear, however, to what extent gene expression in peripheral blood reflects gene expression changes in the brain, and ultimately whether peripheral blood can function as a neural probe (Chana et al., 2013).

There are limitations in the present study. First, we specified relatively low cut-offs and waived duration criteria in defining affective states. It is possible that in these patients where *PTGDS* and *AKR1C3* mRNA expression was down-regulated in both a manic/hypomanic and a depressive state as well as a euthymic state, alterations between depressive and manic/hypomanic states would only be present in more severe episodes. Second, it is possible that state-related alterations in the mRNA expression of *PTGDS* and *AKR1C3* could be observed in drug-naïve patients. It is, however, likely not feasible to include a large cohort of unmedicated rapid-cycling bipolar disorder patients, due to the severity of illness of these patients. Third, our sample size was relatively modest, and given the naturalistic design, not all patients experienced episodes of all polarities and the contribution of between-subject variation was therefore relatively large. Since the number of hypotheses tested was relatively small and hypotheses, outcomes, and covariates were specified *a priori* and other tests were treated as hypothesis-generating, we did not correct statistical analyses for multiple testing, which may be considered appropriate (Streiner and Norman, 2011; Panda et al., 2013) but may be regarded as a limitation. Finally, the included rapid-cycling bipolar disorder patients had relatively long durations of illness and it is possible that the prolonged illness courses could have induced sustained down-regulation of these enzymes to a degree where more subtle expression changes between affective episodes do not occur. While the study could possibly suggest that *PTGDS* mRNA down-regulation in bipolar disorder patients is trait related, it is thus possible that the expression pattern could differ in patients with a more benign course or in patients in early stages of the disorder. Therefore it would be of interest to study mRNA alterations in a larger cohort including bipolar disorder patients in early, intermediary, and late stages of the disorder.

Conclusion

In conclusion, we demonstrated down-regulation of mRNA expression of *PTGDS* in depressed, manic/hypomanic, and euthymic states in rapid-cycling bipolar disorder patients compared with healthy control subjects, with no alterations between affective states. The results suggest a role for aberrant regulation of *PTGDS* and of AA cascade and prostaglandin pathway dysregulation in rapid-cycling bipolar disorder. Further investigation of the AA cascade as a potential therapeutic target is warranted.

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Statement of Interest

Lars Vedel Kessing has within the preceding three years been a consultant for Lundbeck and AstraZeneca.

Maj Vinberg has been a consultant for Lundbeck, AstraZeneca, Eli Lilly and Servier.

Klaus Munkholm and Lone Peijs declare no conflict of interest.

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