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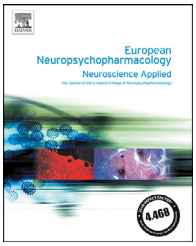

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Genes, biomarkers, and clinical features associated with the course of bipolar disorder

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Abstract
There is considerable variability in the severity of bipolar disorder, e.g., in terms of the frequency of inpatient episodes. The long-term progression also differs, where some patients are sensitised with progressively shorter healthy intervals. Little is known about the proportion of patients being sensitised, their clinical characteristics, and biological underpinnings. We analysed long-term progression of bipolar disorder in relation to clinical characteristics ($N = 3074$), serum biomarkers ($N = 745$), and genetic variants ($N = 1401$) in a cohort of Swedish bipolar disorder patients. We took advantage of the National Patient Register, providing reliable data on 35,973 psychiatric inpatient care episodes in Sweden since 1973. First, one third of the cohort cluster together with a maximum of one inpatient episode per year, while the remaining two thirds had >1 episode per year. These groups did not differ with respect to clinical features or biomarkers. Second, among patients with at least five inpatient episodes (defined as severely ill), we find one group with progressively shorter cycle-lengths (one fifth of the total cohort, $N = 550$). Compared with those with a stable or recuperant trajectory, these patients featured lower functioning, more antidepressant treatment, as well as reduced levels of inflammatory markers in serum. Third, sensitisation was associated with a common genetic variant near the calcium channel gene *CACNA2D3* at genome-wide significance. These results suggest the potential for translational research aimed at preventive actions.

1. Introduction

Prototypical bipolar disorder has a recurrent course with manic and depressive episodes interspersed with euthymic periods. Kraepelin observed in 1921 that the intervals

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between two mood episodes – the cycle length – decrease as a function of the number of episodes (Kraepelin, 1921). According to this observation, bipolar disorder would progressively worsen over time with ever shorter duration of euthymia after each mood episode.

This phenomenon has been confirmed in many – but not all – studies (Kessing and Andersen, 2017). For example, Danish register studies investigated recurrence in terms of hospital admissions and observed a progressive course of bipolar disorder (Kessing et al., 1998b) irrespective of age or sex (Kessing, 1998; Kessing et al., 1998a). It should be noted, however, that some studies did not correct cycle length for the number of elapsed episodes. Failing to do so will give the false appearance that the interval shortens at the group level, simply because patients with shorter cycle length have more episodes than patients with longer cycle length. This has been called Slater's fallacy (Oepen et al., 2004). Considering this, Kessing et al. analysed a Swiss cohort of 220 severely ill patients with bipolar disorder using frailty models, but found no dramatic effect of this adjustment (Kessing et al., 2004).

Post and colleagues suggested a sensitisation model for affective disorder where the episodes become more frequent, more severe, and less triggered by stress-related events over time (Post et al., 1986). The *kindling hypothesis of bipolar disorder* would – at least in theory – be analogous to the sensitisation effect seen in epilepsy, where repetitive electrical stimulation of amygdala in rodents gradually leads to generalised seizures (Goddard, 1967), and even spontaneous seizures at a late stage. The sensitisation model was tested in a small Swedish study using retrospective life charting from thirty patients with treatment refractory depression (Ehnvall and Agren, 2002), where two-thirds of patients showed a sensitisation course whereas one third showed no sensitisation.

Taken together, current evidence suggest that the cycle length decreases during the course of illness for some but not all bipolar disorder patients. However, little is known about potential distinguishing clinical features of patients with decreasing and increasing cycle length, respectively. Further, although Post hypothesised that decreased levels of BDNF might negatively affect the course of illness (Post, 2007), the research on biomarkers in relation to the kindling phenomenon has been scarce, and there are no studies analysing the genetic underpinnings of sensitisation. As bipolar disorder is highly hereditary (Pettersson et al., 2019), one may speculate that the sensitisation phenotype would have biological underpinnings mirrored in genotype variants or serum biomarkers.

The aim of this study was to analyse long-term progression of bipolar disorder in relation to clinical characteristics, serum biomarkers, and genetic variants.

2. Experimental procedures

2.1. Subjects

Patients in this study were recruited for a genetic study: the Swedish Bipolar Collection (SWEBIC, 2009–2013). Cases were mainly enrolled from the Swedish National Quality Register for Bipolar Disorder (BipoläR), but also via the St. Göran project (a long-term follow-up program at the bipolar outpatient unit at the Northern Stockholm psychiatric clinic, Stockholm, Sweden (Ekman et al., 2010; Jakobsson et al., 2013; Ryden et al., 2009)), as well as from the National Patient Register. The quality register BipoläR contains data on individual patients regarding diagnoses (bipolar disorder type 1, type 2, not otherwise specified, or schizoaffective syndrome bipolar type), sociodemographics, interventions, and clinical outcomes (e.g., Global Assessment of Functioning / symptom (GAF-S) and functioning (GAF-F) and Clinical Global Impression- severity of illness (CGI-S)) (Karanti et al., 2015; Tidemalm et al., 2014). Subjects that were included in the BipoläR (82% of the entire cohort) were followed up annually. We use the values from GAF-F/S and CGI-S from the most recent registration in BipoläR. For the purpose of SWEBIC, study nurses conducted a complementary telephone interview with all study subjects. Blood samples were collected for all subjects and stored in −80°C in Karolinska Institutet Biobank pending analyses.

Study data was then linked to the National Patient Register using the unique individual Swedish national registration numbers assigned to each individual at birth or upon immigration. The National Patient Register includes all inpatient admissions since 1973, and each admission has a discharge diagnosis according to the ICD 8, 9 and 10 (International Classification of Diseases) system. The current register linkage includes all admissions up to 2016. See Supplementary material for details regarding diagnoses.

The project was approved by the Regional Ethical Review Board in Stockholm, Sweden, and all participants provided written informed consent.

2.2. Statistical analysis

Unless otherwise stated, analyses were performed using custom-made scripts in MATLAB (MathWorks R2017b). Hypothesis testing was done using Student's *t*-test for numerical data, and Fisher's exact test or Chi-square test for dichotomous data. Chi-square tests were performed in SPSS (IBM Version 25). The false discovery rate method was used to control for multiple testing in the biomarker analysis (Benjamini and Hochberg, 1995), whereas Bonferroni correction was used for clinical characteristics. For the biomarker analysis, a threshold q value of 0.20 was used, meaning that 20% of statistically significant values are possible false positives.

2.3. Time line metric

We used two different metrics for the longitudinal analysis (Supplementary Fig. 1). The first was the number of inpatient episodes per year, where trends were pairwise compared using dynamic time warping with the in-built function *dtw* in MATLAB (Sakoe and Chiba, 1978). The results were used to calculated the similarity between each time-line yielding a distance matrix. In this technique, two signals to compare are stretched in time so that the sum of Euclidean distances is minimised. This method was originally used for speech recognition, where one signal may be very similar to another but either accelerated or decelerated, and standard cross-correlation methods for comparisons will fail to detect genuine similarities. Dynamic time warping gives a pairwise distance between all signals and this information is collected in a distance matrix. Next, the patients were clustered according to the logarithm of the distance matrix by employing hierarchal clustering (with a maximum of eight clusters). The second metric concerned time dependence of cycle-lengths. If sensitisation occur, the cycle-length decreases with time, meaning that episodes occur more and more frequently. Conversely, patients with no sensitisation have either constant or progressively longer interval between hospital admissions. Individual trends were estimated by

fitting a linear curve for the cycle-length as a function of number of previous episodes, whereafter the slope was used to separate patients with sensitisation from those without sensitisation.

2.4. Serum biomarkers

Serum samples were available from 745 patients from year 2009 to 2013. A total of 203 different protein biomarkers were measured using proximity extension assay during year 2015 (Lundberg et al., 2011). The biomarkers were part of three sets of predetermined proteins common in oncology, inflammation, and cardiovascular disease. Functional enrichment of proteins was analysed using STRING (Szklarczyk et al., 2017). We did not attempt to correct for possible confounders (smoking, medication etc.), as we did not have values for both biomarker levels and confounders for a majority of the cases.

2.5. Genomic analyses

We conducted a genome-wide association study (GWAS) for disease progression (where the slope of the fitted curve for the cycle-length was used as dependent variable) in the 1401 patients who had at least five inpatient episodes. DNA was extracted from whole blood samples at the Karolinska Institutet Biobank (mentioned above). Genotyping was conducted in three waves at the Broad Institute of Harvard and MIT using Affymetrix 6.0 (Affymetrix, Santa Clara, CA, USA), Illumina OmniExpress chips (Illumina, San Diego, CA, USA), and the Infinium PsychArray-24 (Illumina, San Diego, CA, USA). Quality control steps were conducted using the Ricopili pipeline. The quality control exclusionary measures for subjects were: genotype missingness rate >5%, ancestry outliers identified via multidimensional scaling (MDS), suspected sample error or contamination (i.e., subject heterozygosity rate >10%), ambiguous genetic sex, and a randomly selected member of any pair of subjects identified as related (pi-hat > 0.20). Exclusionary measures for single nucleotide polymorphism (SNPs) were: marked deviations from Hardy-Weinberg equilibrium ($P<1\times10^{-6}$), SNP missingness rate >5%, minor allele frequency (MAF) <1%, differential missingness based on affection status ($P<1\times10^{-6}$), and differential missingness based on haplotype ($P<1\times10^{-10}$). Following the quality control steps, imputation was performed by first pre-phasing the data using SHAPIT2 and then imputing using PBWT (Positional Burrows-Wheeler Transform) methods with the Haplotype Reference Consortium (HRC) reference panel. All analyses were performed using PLINK2 (Purcell et al., 2007). Linear regression was used including covariates for age, sex, and population substructure captured by the first four principal components. Each genotyping wave was analysed separately and results were combined by meta-analysis. The genome-wide significance threshold was set at $p<5\times10$-8 and markers with <1% frequency or with low quality imputation (INFO<0.6) were removed. See reference (Song et al., 2016) for further details. The final analyses across the three genotyping waves included 7561,151 variants tested after imputation. Furthermore, polygenic risk scores were calculated from genome-wide genotype data as previously described (Song et al., 2016; Stahl et al., 2019). Scores for bipolar disorder and schizophrenia were calculated for 5128 patients. See Supplementary material for more details.

3. Results

3.1. Subjects

The original data set contained 5476 patients, whereof 4663 subjects (85.2%) had at least one discharge from a psychiatric ward. To estimate risk of recurrence, we restricted the analysis to the 4032 subjects with at least two inpatient episodes.

3.2. Recurrence

We considered new inpatient episodes within eight weeks after discharge as relapse in the same episode rather than a new episode (Kessing et al., 1998b). Recurrent episodes were thus separated by at least eight weeks. With this definition, the number of patients with at least two inpatient episodes decreased to 3074 (Supplementary Table 1).

3.3. Survival analysis

To study the general trend in time for cycle-length at the group level, we performed a survival analysis (time in remission). Fig. 1(A) shows that cycle-length decreases with the number of previous episodes. For example, after the first episode, around 27% of the patients are in remission after five years. By contrast, after the 10th or 20th episode, only around 8% and 6%, respectively, are in remission after five years. To circumvent selection bias (i.e., Slater's fallacy), we separately analysed patients with at least five inpatient episodes (1667 patients). The survival curves for this subgroup are identical to those of all patients except for the first episode, for which the survival was lower for all years (blue dashed line B compared to blue solid line A). Still, the survival time is shorter for all years after the fifth episode compared to the first episode when restricting the analysis to patients with at least five episodes. However, in order to completely circumvent Slater's fallacy, we further on analysed individual trends in cycle-lengths.

3.4. Clustering

We employed two different methods of clustering for the two metrics (Supplementary Fig. 1). On time-lines with number of episodes per year, we used dynamic time warping to calculate the similarity between each time-line (starting from the year of onset) and to classify patients in two clusters depending on their frequency of recurrence. In the *Low-frequency recurrence* group, subjects have a maximum of one inpatient episode per year (see Supplementary material for more details). Fig. 1(B), shows the number of episodes per year for *Low-frequency recurrence* group compared to *High-frequency recurrence* group (mean and 95% confidence interval). The two clusters are almost identical concerning gender, age at first admission (Supplementary Table 2), despite that *High-frequency recurrence* group had twice as many inpatient episodes, which follows from the definition.

We also clustered patients according to the second metric (cycle-length as a function of the number of previous episodes). Here, we used the upper third (positive) and lower third (negative) for the slope of the fitted linear curve to cluster patients in one *Non-sensitised* and one *Sensitised* group, respectively. We filtered out patients with less than five inpatient episodes in order to analyse long-term progression (defined as severely ill). Fig. 1(C) displays shorter, respectively longer, cycle-length (mean and 95% confidence

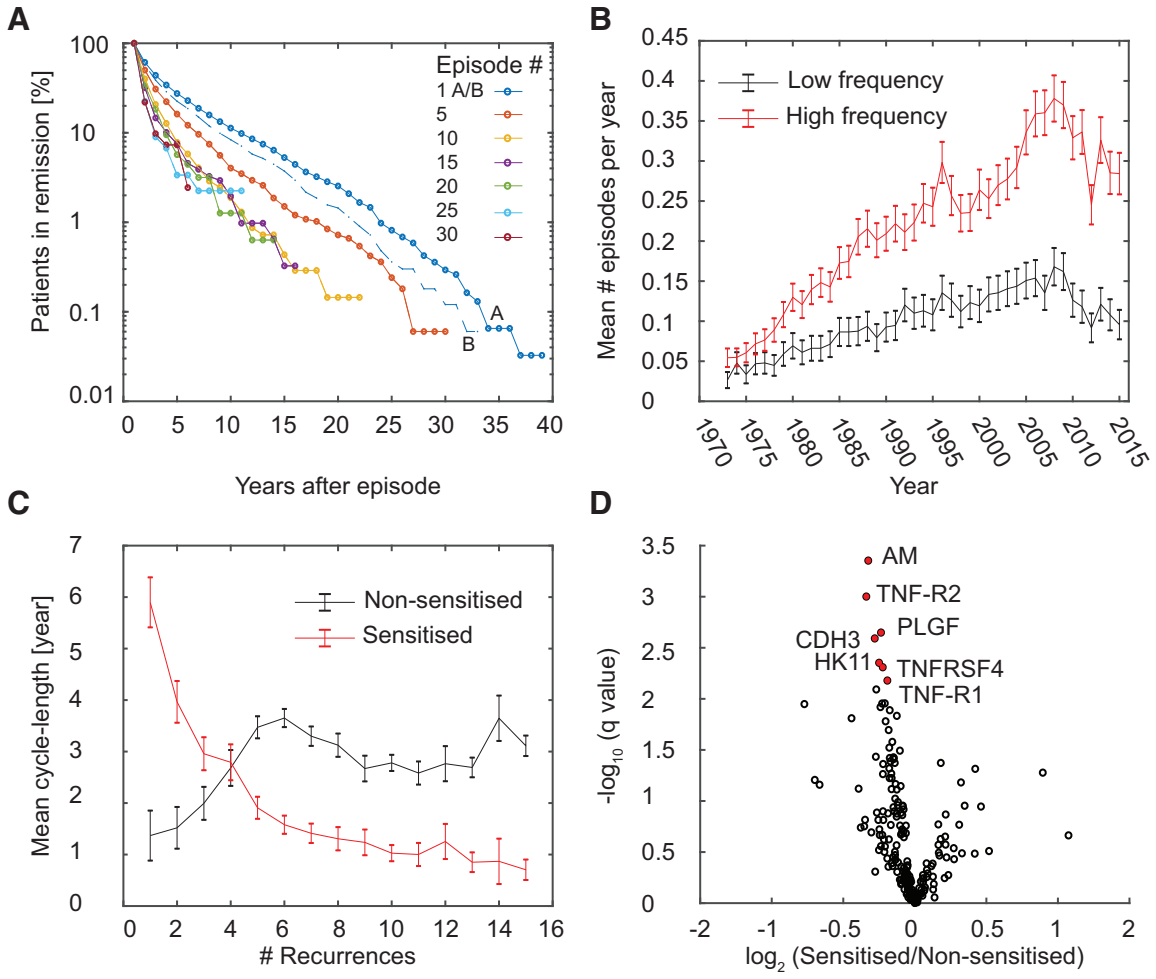

Fig. 1 (A) Survival curves in logarithmic scale for different number of episodes. Curve 1A denotes all subjects and 2B those with at least five inpatient episodes. (B) Mean number of in-patient episodes per year. (C) Mean cycle-length as a function of previous number of inpatient episodes. We did not plot the last relapses because of low numbers of samples. (D) Volcano plot for 203 biomarkers in serum from 60 *Non-sensitised* and 76 *Sensitised* patients. Rings marked in red denote hits with q values less than 0.2. Error bars denote 95% confidence intervals.

interval) when comparing the clusters as a function of number of relapses. Although the two clusters are similar, the *Sensitised* group was slightly younger (Table 1). Interestingly, the mean total number of inpatient episodes did not differ.

3.5. Clinical characteristics

From the BipoläR dataset, we had 20,713 entries for 4793 patients (82% of the entire population cohort). For the first metric, number of inpatient episodes per year, we had data on 756 subjects in the *Low-frequency recurrence* group and 1653 subjects in the *High-frequency recurrence* group. Interestingly, there were no significant differences between these two clusters regarding diagnostic subtype, severity of illness, suicide attempts, or pharmacological treatment (Supplementary Table 3). For the second metric, we had data on 413 patients from the *Non-sensitised* group and 398 patients from the *Sensitised* group. The *Sensitised* group was significantly more ill with lower values on GAF-F and

Table 1 Characteristics of patients in *Non-sensitised* and *Sensitised* groups. Values are mean (SD) for Age at first episode, and Number of episodes.

Group	Non-sensitised	Sensitised	*P* value*
Number	550	550	
Females%	64	61	$p = 0.3824$[1]
Age at first episode	31.6 (10.4)	30.2 (10.7)	$p = 0.0364$[2]
Number of episodes	9.4 (3.9)	9.5 (3.6)	$p = 0.74$[2]
Depressive/manic episode%	28/21	29/27	$p = 0.53$[3]

[1] Fisher's exact test.
[2] Student's *t*-test.
[3] Chi-square test.
* Correction for multiple hypothesis testing: $\alpha_{Bonferroni} = 0.05/4 = 0.0125$.

Table 2 Clinical characteristics of patients in the *Non-sensitised* and *Sensitised* groups.

Group	Non-sensitised	Sensitised	*P* value*
Number	413	398	
Bipolar subtype: 1/2/NOS/SAD-BD %[4]	65/16/14/4	59/24/14/3	$p=0.57$[1]
BMI[4]	28 (6.0)	29 (6.0)	$p=0.27$[2]
Smokes %[7]	11	18	$P=0.0056$[3]
Heredity %[4,5]	39	37	$p=0.56$[3]
GAF-F[6]	**66 (14)**	**62 (14)**	$\mathbf{P=0.0002}$[2]
GAF-S[6]	**67 (14)**	**63 (14)**	$\mathbf{P=0.00014}$[2]
CGI-S%[6]	**3.1 (1.6)**	**3.7 (1.6)**	$\mathbf{P=0.000006}$[2]
Depressive episode: 0/1-3/4+%[4]	6/14/70	3/16/69	$p=0.57$[1]
Hypomanic episode: 0/1-3/4+%[4]	21/24/44	19/25/43	$p=0.95$[1]
Manic episode: 0/1-3/4+%[4]	22/24/43	25/28/35	$p=0.52$[1]
Mixed episode: 0/1-3/4+%[4]	50/21/18	51/15/22	$p=0.55$[1]
Attempted suicide%[4]	42	40	$p=0.57$[3]
Age first symptom: 0-7/8-11/12-17/18-24/25- years old[4]	0.7/4.1/14/19/18	2.2/3.8/15/15/18	$p=0.94$[1]
Psychiatric comorbidity%[4]	16	21	$p=0.15$[3]
Mood stabilizer%[4]	87	84	$p=0.23$[3]
Lithium%[4]	70	60	$p=0.0041$[3]
Antidepressant%[4]	**38**	**52**	$\mathbf{p=0.000074}$[3]
Antipsychotic%[4]	44	51	$p=0.049$[3]
Benzodiazepine%[4]	33	40	$p=0.029$[3]

NOS not otherwise specified. SAD=Schizoaffective disorder bipolar type. Global Assessment of Functioning/symptom (GAF-S) and functioning (GAF-F). CGI-S= Clinical Global Impression- severity of illness.

[1] Chi-square test.
[2] Student's *t*-test.
[3] Fisher's exact test.
[4] Value at first registration.
[5] Subjects reporting any first or second degree relative with a mood disorder.
[6] Value at last registration.
[7] Value on any occasion.
* Correction for multiple hypothesis testing: $\alpha_{Bonferroni}=0.05/19=0.0026$.

GAF-S and higher values on CGI-S (Table 2). There were fewer patients treated with lithium, but more patients treated with antidepressants, antipsychotics and benzodiazepines in the *Sensitised* group. Notably though, the differences regarding lithium, antipsychotic and benzodiazepine use did not survive multiple testing correction. For 4965 patients (85% of the entire cohort), we also had data from telephone interviews on subjective treatment response to lithium and autoimmune diseases, but found no significant differences between the clusters (See Supplementary Tables 4 and 5).

3.6. Biomarkers

We analysed possible biomarkers for differences between the two groups. First, comparing *Low-frequency recurrence* group with *High-frequency recurrence* group yielded no significant differences (samples available for 98 and 242 patients, respectively). Likewise, we compared biomarker levels between the *Non-sensitised* and *Sensitised* groups (samples available for 38 and 47 patients, respectively). Four proteins were expressed at significantly lower level in the *Sensitised* group at $q < 0.20$. These were TNFR2 (Tumor necrosis factor receptor 2, $q=0.10$), TNFRSF4 (Tumor necrosis factor receptor superfamily member 4, $q=0.17$), PLGF (Placenta growth factor, $q=0.13$), and AM (Adrenomedullin, $q=0.090$). To increase sample size, we split patients with regards to positive or negative slope (see above). With 60 and 76 patients, respectively, seven proteins differed significantly between the groups (Fig. 1(D)). In addition to the four proteins above, CDH3 (Cadherin-3, $q=0.13$), KLK11 (Kallikrein-11, $q=0.17$), and TNFR1 (Tumor necrosis factor receptor 1, $q=0.19$) were expressed at a lower level in the group with negative slopes. See Supplementary File 1 for complete data. We detected functional enrichment in the biological processes *Tumor necrosis factor-mediated signalling pathway* ($q=0.00167$), the molecular function *Tumor necrosis factor-activated receptor activity* ($q=8.32\times10^{-6}$), as well as the KEGG pathway *Cytokine-cytokine receptor interaction* ($q=0.0175$).

3.7. GWAS

Next, we performed a GWAS on 1401 patients with at least five inpatient episodes with the slope of fitted curve as dependent variable. The C/T SNP rs141975312 on chromosome 3 close to *CACNA2D3* turned out genome-wide significant (β=0.001768, $p=1.038\times10^{-8}$ and MAF 1.5%), see Fig. 2(A) for Manhattan plot. The regional plot (Fig. 2(B)) shows that neighbouring SNPs also demonstrated a nominal association. There was no evidence of inflation in the QQ-plot (λ=99.6%, Supplementary Fig. 2(D)). The second nominally significant

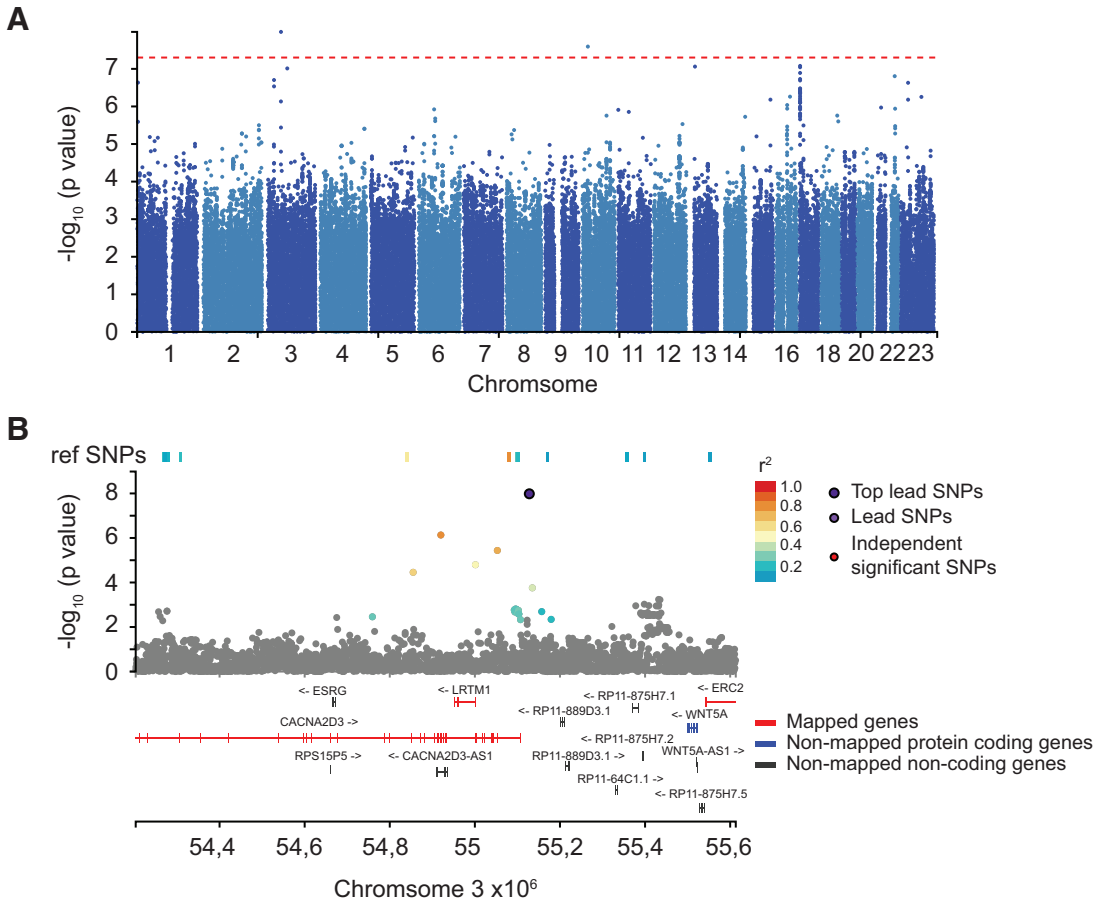

Fig. 2 (A) Manhattan plot for GWAS results with a SNP at chromosome 3 demonstrating genome-wide significance. (B) Regional plot for the association signal on chromosome 3.

SNP at chromosome 10 was rejected after controlling for age, sex, and population substructure.

3.8. Polygenic risk scores

We calculated polygenic risk scores for schizophrenia and bipolar disorder for in total 5128 patients. When comparing all patients included in the different clusters, we could not find any significant differences in the mean polygenic risk scores between the four clusters. *Low frequency recurrence* ($N = 847$) vs. *High frequency recurrence* ($N = 1856$) groups (Schizophrenia: 0.16 (0.98) vs. 0.16 (0.97), $p = 0.86$ and Bipolar: 0.26 (0.96) vs. 0.25 (0.98), $p = 0.71$). *Sensitised* ($N = 481$) vs. *Non-sensitised* ($N = 475$) groups (Schizophrenia: 0.26 (0.98) vs. 0.19 (0.89), $p = 0.30$ and Bipolar: 0.33 (1.0) vs. 0.34 (0.96), $p = 0.97$). Also, there was no correlation between risk scores and slope of fitted curve (Schizophrenia: $R^2 = 2.6^{*}10^{-6}$ $p = 0.95$ and Bipolar: $R^2 = 7.8^{*}10^{-4}$ $p = 0.29$).

4. Discussion

We analysed the variability in long term progression of bipolar disorder by studying the clinical trajectory of individual subjects, thereby circumventing the problem with Slater's fallacy. We took advantage of the National Patient Register, providing reliable data on 35,973 psychiatric inpatient care episodes in Sweden since 1973 (Ludvigsson et al., 2011). We first conducted a cluster analysis by counting the number of inpatient episodes per year. The *Low-frequency recurrence* group contained 32% of all eligible patients, whereas the *High-frequency recurrence* group contained 68% of all patients. Second, we separated patients with at least five hospital admissions based on whether their cycle-length decreased (*Sensitised* group; 33%) or increased (*Non-sensitised* group; 33%).

We found no clear differences with respect to clinical or biological characteristics between the *Low-frequency* and *High-frequency recurrence* groups, except for the number of inpatient episodes that followed from the definition. By contrast, patients in the *Sensitised* group, were significantly more ill in terms of GAF and CGI, and were more often treated with antidepressants, than the *Non-sensitised* group. Also, patients in the *Sensitised* group were, at least nominally, less often treated with lithium, but more often with antipsychotics and benzodiazepines. Self-reported lithium response did not, however, differ between the groups. Also, the groups did not differ in terms of psychiatric comorbidities or the number suicide attempts.

In an exploratory analysis with a rather liberal *q* value threshold (0.2), we compared the *Sensitised* group with the *Non-sensitised* group with respect to 203 serum biomarkers. We identified seven proteins that were expressed at a lower level in the *Sensitised* group compared to the *Non-sensitised* group. First, we identified TNFR1 and TNFR2 that are the two distinct receptors via which TNF-α imposes its proinflammatory effects. Interestingly, the soluble form of the TNF-α receptor 1 has consistently been found to be increased in the blood of bipolar disorder patients (Modabbernia et al., 2013). Also, mood-disorder patients have been found to have decreased protein levels of TNFR2 in dorsolateral prefrontal cortex, which is known to be involved in mood regulation (Dean et al., 2013). TNFRSF4 is expressed on activated T-cells and is thought to prolong the immune response (Song et al., 2005). PLGF has a role during pregnancy but is also involved in inflammation in atherosclerotic lesions (Khurana et al., 2005) and possibly in post-stroke depression (Yue et al., 2016). Adrenomedullin is a peptide hormone thought to regulate vasodilatation, but has also been shown to be upregulated in plasma of bipolar disorder patients (Savas et al., 2002). Interestingly, the concentration of adrenomedullin in plasma was negatively correlated with total duration of illness in this cohort of patients. In sum, the sensitised group of patients was characterised by lower serum levels of inflammatory molecules than the non-sensitised group. Importantly, there was no difference in the prevalence of autoimmune diseases among the different groups of patients (Supplementary Table 5).

Several previous papers have reported on long-term progression of bipolar disorder and some researchers discuss the possible role of neuroprogression, where patients progressively develop cognitive and functional impairment during the course of illness (Berk, 2009). In one study, the authors compared levels of inflammatory molecules as well as BDNF between patients in early and late stage bipolar disorder and found that BDNF was lower only at the late stage (Kauer-Sant'Anna et al., 2009). By contrast, the pro-inflammatory molecules TNF-α and IL-6 were increased at both early and late stages, whereas the anti-inflammatory molecule IL-10 was increased only at the early stage. These data suggest that late stage bipolar disorder is characterized by a pro-inflammatory drive. In our data, both the *Sensitised* and *Non-sensitised* patients had at least five inpatient hospitalizations for mood episodes and their first inpatient episodes on average around 20 years ago. Interestingly, the *Sensitised* patients, a subgroup among late stage bipolar disorder, is characterised by lower levels of inflammatory molecules in serum, including three different receptors for TNF-α.

Considering polygenic risk scores for bipolar disorder and schizophrenia, we could not detect any significant differences between the different clusters. Also, there was no correlation between risk scores and slopes of fitted curves, i.e., the polygenic risk scores was not associated with whether recurrences occurred more or less often over time. However, in the GWAS we detected a significant association for sensitisation with rs141975312, a common variant near *CACNA2D3*. This gene encodes the alpha 2/delta subunit 3 of voltage-gated calcium (Ca^{2+}) channels. These channels are comprised of four subunits and mediate influx of Ca^{2+} upon membrane depolarization in neurons. The alpha 2/delta subunit 3 regulates the activity of for instance L-type channels. Although this specific gene has not been associated with psychiatric illness before, Ca^{2+} channels in general have been strongly implicated in the pathogenesis of several psychiatric disorders, including bipolar disorder (Heyes et al., 2015). *CACNA1C* in particular, encoding the alpha 1c subunit of L-type channels, has been repeatedly associated with bipolar disorder (Cross-Disorder Group of the Psychiatric Genomics, 2013; Ferreira et al., 2008; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011).

4.1. Limitations

This cohort study defined disease activity as inpatient episodes at psychiatric clinics. Thus, depressive and hypomanic episodes that are managed in an outpatient setting are not captured. It is not clear how this might affect the results, although it most certainly means that the burden of disease is underestimated. Furthermore, the relatively small number of patients that were available for biomarker analysis in serum reduces the power to find conclusive and statistically strong results. In these exploratory analyses, we decided to use a threshold value of false discovery rate at 20% in order to increase the chance of identifying possible differences. It is not obvious that these results would survive the analysis from a larger sample. Also, due to lack of information we were not able to correct for smoking or medication. This is relevant, as for example treatment with antipsychotics significantly impacts levels of inflammatory markers in cerebrospinal fluid (Isgren et al., 2015).

5. Conclusion

By analysing the time-course of individual patients we circumvented Slater's fallacy and identified one group constituting around one fifth of the cohort that have a deteriorating course of illness with progressively shorter cycle-length. This group had lower level of functioning, more often treatment with antidepressants, and had lower serum levels of inflammatory markers. Also, the measure of sensitisation was associated with a SNP in the Ca^{2+} channel subunit gene *CACNA2D3*.

Role of the funding source

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Contributions

Erik Smedler designed the study and did most of the analyses as well as wrote the first draft of the paper. Sarah Bergen performed the genome-wide association study. Jie Song performed the polygenic risk score analysis. Mikael

Landén supervised and financed the project. All authors contributed to and have approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.07.132.

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