PCN Psychiatry and Clinical Neurosciences

REGULAR ARTICLE

Polygenic score for schizophrenia worsens the effect of bipolar disorder course on neuronal metabolism and white matter microstructure

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Aim: Bipolar disorder (BD) and schizophrenia (SCZ) share many clinical and neurobiological features, and a continuum between the two has been postulated. Bipolar patients leaning toward the SCZ pole of the continuum may have a higher risk of neuroprogression. Here we investigated the relationships between illness course, white matter integrity, levels of N-acetylaspartate (NAA), and polygenic score (PRS) of SCZ.

Methods: A sample of 103 depressed bipolar inpatients underwent magnetic resonance imaging (MRI) acquisition to perform diffusion tensor imaging (DTI) analysis and magnetic resonance spectroscopy to assess NAA. Genotyping and PRS calculation were also performed in a subsample of 75 patients. Associations between illness course, NAA, and white matter microstructure were explored; indirect effects were investigated through mediation models; further, a possible moderating effect of SCZ-PRS was tested.

Results: Negative associations emerged between number of affective episodes and NAA. Manic episodes were also negatively associated with white matter integrity, and NAA significantly mediated the effect of manic episodes on DTI metrics. SCZ-PRS moderated the relation between illness duration and NAA. Moderated mediation analyses showed that only at high SCZ-PRS, illness duration negatively affected NAA, which in turn was linked to reduced fractional anisotropy.

Conclusion: Our results support the concept of neuroprogression in BD, suggesting a deleterious effect of acute episodes, particularly manic ones, on neurochemical and white matter alterations. Further, patients with a higher SCZ-PRS seem to show detrimental effects related to illness duration, possibly suggesting a longitudinal course closer to SCZ.

Keywords: *bipolar disorder, N-acetylaspartate, neuroprogression, schizophrenia polygenic score, white matter.*

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The modern concept of bipolar disorder (BD) comes from the foundational work of Kraepelin at the end of the 19th century, when he distinguished manic-depressive insanity from dementia precox (later renamed schizophrenia, SCZ), identifying one crucial discriminating element in *restitutio ad integrum*, that is, full recovery, characteristic of BD but not SCZ.[1] However, Kraepelin himself reported cases with intermediate clinical pictures and no clear boundary between the two disorders.[1,2]

Consensus now is that BD and SCZ share some features, with significant overlaps in terms of symptoms, familial patterns, genetics, course, outcome, and treatment[1,3]; indeed some authors propose a continuum, with prototypical SCZ and BD as the two opposite poles and many intermediate conditions in between.[1]

While a feature historically attributed to SCZ is its degenerative nature, it has become clear that a portion of bipolar patients exhibits a progressive course of illness with a worsening clinical picture, chronicization of symptoms, and functional deterioration.[4,5] As the illness progresses, these patients tend to present more frequent and longer recurrences, with more severe symptoms, shorter euthymic periods, and residual inter-episodic subsyndromal symptoms.[4,6,7]

The term neuroprogression has been introduced to define the longitudinal brain changes that underlie this progressive clinical and functional deterioration.[8] Longitudinal and cross-sectional evidence indicates that neuroprogressive changes in BD are strongly related to the occurrence of (hypo)manic episodes.[9–11] In a large longitudinal study by the ENIGMA consortium, bipolar patients who experienced (hypo)manic episodes in the follow-up period showed cortical thinning in the frontal lobe, anterior cingulate cortex (ACC) and medial occipital cortex, in contrast with patients who did not experience manic episodes, who showed no changes or increased cortical thickness in these areas.[12] Moreover, the number of (hypo)manic or mixed episodes correlated with the magnitude of the changes reported.[12]

While neuroprogression has been extensively studied in relation to gray matter volumes and cortical thickness, fewer authors have investigated other neuroimaging modalities. Diffusion tensor imaging (DTI) studies of WM microstructure are limited, investigate heterogeneous samples, and tend to report inconsistent results.[13–18] A few

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studies found an association between illness duration or the number of mood episodes and a decline in WM integrity in bipolar patients,[13,16–18] and other studies comparing bipolar patients and healthy controls found a significant age-by-group interaction on fractional anisotropy (FA), showing that bipolar patients have a faster age-related WM deterioration than healthy individuals.[15,16]

Even fewer studies explored the association between illness progression and brain metabolites levels, assessed by proton magnetic resonance spectroscopy (H1-MRS). Among brain metabolites, N-acetylaspartate (NAA) might be particularly relevant in the context of BD neuroprogression: is one of the most abundant metabolites in the human brain, and the most prominent signal in brain spectroscopy[19]; while its exact function is still somewhat controversial, its levels are commonly interpreted as an indicator of neuronal and axonal health and integrity, due to its crucial role in neuronal metabolism and myelination, and are found to be reduced in several brain pathological conditions.[20] While research on NAA levels along the course of BD is limited and heterogeneous, some results converge with structural evidence supporting a progressive decline of its levels.[21–23] One study reported a significant negative correlation between NAA and illness duration in patients not treated with lithium, while treated patients did not show such correlation, highlighting the neuroprotective role of lithium in BD.[23] Another study found a correlation between NAA and the number of previous hospitalizations for mania, but not with the duration of illness or the number of depressive episodes, further supporting the specific role of mania in neuroprogression.[22]

Following this line of reasoning, we aim to test the effect of clinical history indicators—that is number of affective episodes and duration of illness—on brain metabolites and WM microstructure, also testing potential indirect effects. Further, considering that higher polygenic liability for SCZ has been consistently associated to worse psychotic symptoms, worse treatment outcomes, reduced inter-episode remission, and lower GAF-function in bipolar patients,[24–29] we aim to test the potential role of SCZ genetic liability in modulating the relation between clinical history and neuroimaging biomarkers. We hypothesize that longer duration of illness and a higher number of previous episodes will associate with brain features suggestive of neuroprogression (such as reductions in NAA levels and FA) and that such associations will be more pronounced in individuals with a higher SCZ genetic liability.

Methods

Participants

Our sample was composed of 103 patients with a diagnosis of BD (age range 20–69), consecutively admitted to the mood disorder ward of San Raffaele Hospital in Milan, during an ongoing depressive episode. Each patient's diagnosis was made by staff psychiatrists according to DSM-5 criteria. Exclusion criteria were current diagnosis of any additional psychiatric disorder, including alcohol and/or substance dependence in the last 6 months, intellectual disability, pregnancy, major medical, and neurological disorders. All patients underwent 3 T magnetic resonance scanning. A subsample of 75 patients underwent venous blood sampling for genotyping. Patients' duration of illness and number of affective episodes were retrieved from clinical charts. Previous months of lithium treatment were obtained from the clinical history of each patient. After a complete description of the study, written informed consent was obtained. All research activities have been approved by the local Ethical Committee.

Magnetic resonance imaging (MRI) acquisition

Magnetic resonance imaging (MRI) scanning was performed on a 3.0 Tesla scanner (Ingenia CX, Philips, The Netherlands) using a 32-channel sensitivity encoding (SENSE) head coil. A structural MRI study was performed first to rule out brain lesions and to localize the volume of interest (VOI) for the spectroscopy study, acquiring T1 images, T2 fast spin-echo (FSE), and coronal fluid-attenuated inversion recovery (FLAIR) images. T1 and T2-weighted FLAIR images were visually inspected as part of the quality check procedure.

Each subject underwent proton magnetic resonance spectroscopy (^{1}H-MRS). A point-resolved spectroscopy (PRESS) sequence was used for data acquisition with the following parameters: a repetition time (TR) of 2000 ms, an echo time (TE) of 42 ms, and 128 acquisitions. An unsuppressed water reference spectrum was also acquired. A trained radiological technician positioned a 30 × 15 × 15 mm VOI in the left hemisphere to encompass the dorsolateral prefrontal cortex and the ACC. The left hemisphere was chosen in accordance with the majority of studies in the literature to ensure data comparability. The placement of the VOI was guided by anatomical T2-weighted fast spin-echo (T2-FSE) reference images, following these steps: considering the axial section, the voxel was positioned parallel to the central fissure in the left hemisphere; the long side of the voxel was then aligned along the body of the corpus callosum, with its edge beginning at the anterior end of the genu; finally, the voxel was adjusted to exclude the underlying ventricular cerebrospinal fluid (CSF) in the coronal section. VOI placement can be seen in Figure S1.

Diffusion-weighted imaging (DWI) was executed using SE Eco-planar imaging with the following parameters: TR/TE = 5900/78 ms, FoV (mm) 240 (ap), 129 (fh), 232 (rl); acquisition matrix 2.14 × 2.73 × 2.30; 56 contiguous, 2.3-mm thick axial slices reconstructed with in-plane pixel size 1.88 × 1.88 × 2.30 mm; SENSE acceleration factor = 2; 1 b0 and 40 non-collinear directions of the diffusion gradients; b value = 1000 s/mm^2. Fat saturation was performed to avoid chemical shift artifacts.

Data processing and analysis

The ^{1}H-MRS spectra were analyzed using LCModel (version 6.3.0).[30] The analysis utilized a basis set from Provencher, which was specifically tailored to our acquisition sequence and included the following metabolites: alanine, aspartate, creatine (Cr), phosphocreatine (PCr), glucose, glutamine (Gln), glutamate (Glu), glycerophosphocholine, phosphocholine, myo-inositol (mI), lactate, NAA, N-acetylaspartylglutamate, scyllo-inositol, and taurine, as well as signals from macromolecules and lipids. The LCModel software operates by fitting the *in vivo* spectrum as a linear combination of model *in vitro* spectra from individual metabolites. This approach uses complete model spectra, rather than single resonances, to incorporate maximum prior information into the analysis. A constrained regularization method is automatically applied to account for the baseline and lineshape without imposing restrictive models. LCModel automatically handles baseline and lineshape variations, providing objective estimates of metabolite concentrations and their Cramér-Rao lower bounds (CRLBs). The quantification of metabolites was performed using the unsuppressed water signal from the same VOI as an internal reference, assuming 80% water content in the brain tissue.

Full width at half maximum was 0.043 ± 0.008 Hz (range, 0.031–0.071 Hz), and the signal-to-noise ratio was 23.097 ± 3.350 (range, 15–30).

Brain tissue in the three-dimensional T1-weighted brain images was segmented using the Gannet Co-Register and Gannet Segment functions in the Gannet 3.1 toolbox[31] in SPM12. To account for partial-volume effects, tissue segmentation was performed on the VOI to estimate the proportions of gray matter (GM), WM, and CSF. These tissue fractions were then used to apply subject-specific partial-volume correction within LCModel. Specifically, metabolite concentrations were adjusted based on the water content within the GM and WM fractions, using a correction factor customized for each subject's unique tissue composition in the VOI. To ensure the accuracy of the measurements, only corrected metabolite concentrations with a CRLB below 20% were included in the analyses.

From the available measured metabolites, N-acetylaspartate to creatine ratio (NAA/Cr), a commonly used index of neuronal or

axonal loss or dysfunction,[32,33] was calculated to be entered in further analyses. NAA/Cr was preferred to raw NAA values in order to normalize for non-biological variations and provide a more reliable and clinically comparable measure, as creatine serves as a stable internal reference standard.[32,33]

DTI data were analyzed using the FMRIB Software Library (FSL 6.0; www.fmrib.ox.ac.uk/fsl/index.html).[34] Each DWI volume was affine registered to the T2-weighted $b = 0$ volume using FLIRT. Then, correction for motion between scans and residual eddy-current distortions present in the diffusion-weighted images was performed with Eddy FSL's tool. After brain extraction through the BET tool, least-square fits were performed to estimate the FA, eigenvector, and eigenvalue maps using DTIFIT. Then, axial diffusivity (AD), mean diffusivity (MD), and radial diffusivity (RD) were obtained.

Next, all subjects' volumes were skeletonized and transformed into a common space as used in Tract-Based Spatial Statistics (TBSS).[35] Briefly, all volumes were nonlinearly warped to the FMRIB58_FA template supplied with FSL (http://www.fmrib.ox.ac.uk/fsl/tbss/FMRIB58_FA.html) and normalized to the Montreal Neurological Institute space, by use of local deformation procedures performed by FMRIB's Non-Linear Image Registration Tool (FNIRT). Next, a mean FA image of all subjects was calculated and thinned to create a mean FA skeleton representing the centers of all common tracts. In order to reduce the likelihood of partial voluming in the borders between tissue classes, we applied a threshold (FA >0.20) and binarized the mean FA skeleton. Individual FA values were warped onto this mean skeleton mask. The resulting tract invariant skeletons for each participant were fed into voxel-wise permutation-based cross-subject statistics. Similar warping and analyses were also performed on MD, AD, and RD data.

Genotyping and polygenic risk scores calculation

Blood samples of 43 patients were genotyped using the Infinium PsychArray 24 BeadChip (Illumina, Inc., San Diego), which is a cost-effective, high-density microarray developed in collaboration with the Psychiatric Genomics Consortium and containing 595,427 genetic markers associated with psychiatric disorders. Moreover, 32 patients were genotyped through the Infinium Global Screening Array-24 Kit (Illumina, Inc., San Diego), combined with the Psych Booster. This kit includes 654,027 fixed markers along with an additional 30,000 markers from the Infinium PsychArray-24 BeadChip. QC was conducted with PLINK1.9.[36] Individuals with discrepant genotyped sex information, genotype rate <95%, or outlying autosomal heterozygosity (Fhet > ±0.2) were removed. Markers with minor allele frequency (MAF) < 1%, call rate < 95%, or deviant from Hardy–Weinberg equilibrium at $P < 10^{-6}$ were excluded. Relatedness of participants was checked by excluding individuals with a degree of recent shared ancestry (i.e., identity by descent, IBD) > 0.1875, which is the threshold corresponding to the halfway between third- and second-degree relatives.[37] Finally, European population ancestry of the sample was confirmed with a principal component analysis (PCA), removing individuals whose genotype distribution was deviant from more than 5 standard deviations from the mean of the first two components. After QC, the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html) was used for genotype imputation and polygenic score (PRS) calculation. The post-QC dataset was imputed using the Haplotype Reference Consortium panel (i.e., HRC r1.1 2016) as reference panel, adopting Minimac4 and setting the rsq filter to 0.003. Finally, Schizophrenia PRS (SCZ-PRS) was calculated as the sum of the number of effect alleles at each variant position, weighted for their effect size derived from Gui *et al.*[38] through the pgs-calc tool as implemented by the Michigan Imputation Server.[39]

Statistical analyses

The relation between number of affective episodes, duration of illness and NAA/Cr was tested through general linear models (GLM), entering the former as independent variables, NAA/Cr as dependent variable, and age and sex as covariates. Further, to take into account the specific neuroprotective properties of lithium in BD,[44,45] previous months of lithium treatment were also included among covariates.

For 12 participants the exact number of affective episodes could not be determined from their clinical history, and they were therefore excluded from analyses investigating the effect of lifetime episode number.

The relation between clinical variables, NAA/Cr levels and DTI metrics was tested with voxel-wise DTI analyses using nonparametric permutation-based testing as implemented in Randomize in FSL. All analyses were carried out in the context of GLM entering age, sex, and months of lithium treatment as covariates. Threshold-free cluster enhancement (TFCE) was used to avoid defining arbitrary cluster-forming thresholds and smoothing levels. The data were tested against an empirical null distribution generated by 5000 permutations for each contrast, thus providing statistical maps fully corrected for multiple comparisons across space. Corrected $P < 0.05$ was considered significant.

Mean FA, RD and (MD) values of WM tracts affected by clinical variables and NAA/Cr were extracted to be entered in mediation models.[40] This was achieved by creating binary masks from the voxels where a significant effect on DTI metric was identified and then applying such masks to the FA, RD, and MD skeletonized data.

Mediation and moderation analyses were performed through Hayes' PROCESS macro for IBM SPSS. In all analyses, age, sex, and months of lithium treatment were entered as nuisance covariates.

First, a possible indirect effect of the number of (hypo)manic episodes on DTI metrics they affected through NAA levels was tested in a simple mediation model. A possible moderating effect of SCZ PRS on the relation between clinical variables and NAA/Cr was subsequently tested. Finally, after reviewing results of such analyses, a moderated mediation model was tested, entering duration of illness as independent variable, NAA/Cr as mediator, DTI metrics affected by NAA/Cr as dependent variables, and SCZ PRS as moderator.

Results

Clinical and demographic characteristics of the sample can be seen in Table 1. Women represented 52% of the sample, mean age was 49 ± 13 years, with a mean duration of illness of 20 ± 12 years and a mean lifetime number of affective episodes of 10 ± 8 (Table 1).

Effect of clinical variables on NAA and DTI metrics

Duration of illness did not significantly affect NAA levels. Significant negative associations were, on the other hand, found between NAA/Cr and number of affective episodes ($\beta = -0.23$, $F = 11.10$, $P = 0.001$) (Fig. 1a). This relationship was confirmed for both (hypo) manic ($\beta = -0.24$, $F = 12.64$, $P < 0.001$) (Fig. 1b) and depressive mood episodes ($\beta = -0.18$, $F = 6.10$, $P = 0.015$) (Fig. 1c). Two participants exhibited extreme values for both manic and total affective episodes: Excluding them from the analyses did not significantly affect the results (N. Manic ep.: $\beta = -0.22$, $F = 9.66$, $P = 0.003$; N. Total ep.: $\beta = -0.20$, $F = 7.74$, $P = 0.007$).

No significant association was found between duration of illness and DTI metrics. Again, the number of previous (hypo)manic episodes was negatively associated with FA ($P = 0.032$) (Fig. S2a, Table S1) and positively with RD ($P = 0.022$) (Fig. S2b, Table S1) and MD ($P = 0.025$) (Fig. S2c, Table S1), while no effect was detected for depressive episodes.

Effect of NAA on DTI metrics and mediation analysis

NAA levels had strong effects on DTI metrics: widespread positive association with FA ($P < 0.001$) (Fig. 2a, Table S2) and negative with AD ($P = 0.032$), RD ($P < 0.001$) (Fig. 2b, Table S2), and MD ($P = 0.001$) (Fig. 2c, Table S2) were identified. Mediation analysis revealed significant indirect effects of the number of (hypo)manic episodes on DTI metrics through NAA/Cr levels: This was observed for the association with FA (95% BCa CI [−0.00128, −0.00010])

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Table 1. Clinical and demographic characteristics of the sample

	Whole sample ($n = 103$)	Genetic subsample ($n = 75$)	χ^2/t-test P
Age	49.00 ± 12.63	49.04 ± 12.64	0.983
Sex (M/F)	49/54	32/43	0.516
Education (years)	12.92 ± 3.78	13.08 ± 3.83	0.784
Lifetime lithium (months)	47.51 ± 78.96	53.51 ± 79.15	0.618
Age at onset	28.77 ± 10.86	28.27 ± 11.26	0.761
Duration of illness	20.22 ± 11.62	20.77 ± 11.90	0.759
No. depressive episodes	6.83 ± 5.03	6.80 ± 4.97	0.965
No. manic episodes	3.24 ± 3.87	3.40 ± 4.15	0.807
No. episodes (total)	10.09 ± 7.94	10.21 ± 8.18	0.922
NAA/Cr	1.183 ± 0.110	1.181 ± 0.107	0.917

(Fig. 3), RD (95% BCa CI [0.0000002, 0.0000018]), and MD (95% BCa CI [0.0000001, 0.0000016]). In all cases, the direct path of the moderation remained significant (FA: $b = -0.0024$, $P < 0.001$; RD: $b = 0.0000026$, $P = 0.002$; MD: $b = 0.0000023$, $P = 0.003$).

Schizophrenia PRS interaction and moderated mediation analysis

Schizophrenia PRS significantly moderated the relation between duration of illness and NAA/Cr ($b = -0.006$, 95% CI [−0.012–0.0004], $F = 4.51$, $P = 0.037$) (Fig. 4b), with significant negative associations between the two only for higher PRS values (the Johnson-Neyman output of the moderation revealed significant associations in the upper 40.00% of the PRS distribution), while in patients with lower SCZ-PRS no relation between NAA/Cr and duration of illness was identified.

Moderated mediation analysis revealed a significant indirect effect of duration of illness on FA through NAA/Cr levels only for higher SCZ PRSs (Fig. 4a), meaning that the detrimental effect of illness on FA, mediated by a lower NAA/Cr, was detected only in patients with a higher SCZ-PRS. Furthermore, SCZ-PRS significantly moderated the relation between NAA/Cr and FA ($b = -0.119$, 95% CI [−0.230–0.009], $F = 4.63$, $P = 0.035$) (Fig. 4c), with weaker positive associations between the two at increasing values of PRS.

For all analyses, adding years of education among covariates provided analogous results (see Supporting Information).

Discussion

The present study found the number of illness episodes, and not the duration of illness in itself, to have a detrimental effect on MRI measures of brain homeostasis in patients with BD; the effect of illness duration, on the other hand, was revealed only when taking into account SCZ genetic load.

The negative association between the number of affective episodes and frontal NAA/Cr levels in bipolar patients held true for both the number of depressive episodes and, with a stronger association, for the number of (hypo)manic ones. NAA brain levels, measured through proton magnetic resonance spectroscopy, are classically regarded as an index of neuron and axonal health and viability[19] and as such could be particularly suited to reflect consequences of brain insults. The strong negative association we identified with the lifetime number of affective episodes, particularly manic, seems to point to a cumulative pathological effect of mood states experienced by patients. Indeed, a similar result has been reported by Frye and colleagues, who identified a negative correlation between the number of hospitalizations for mania and NAA concentration in the basal ganglia of bipolar patients.[22] Further, it is of particular interest that NAA levels have been repeatedly associated with cognitive performances in bipolar samples,[41–43] giving further credence to the notion that its levels could be reflective of BD neuroprogression. Despite being performed exclusively in manic patients, a recent study found NAA levels in the anterior cingulate of multiepisode bipolar patients to be lower than in first episode ones.[44]

Further linking NAA levels to BD functional deterioration, a study by Hajek and colleagues showed that NAA levels negatively correlate with illness duration in bipolar patients not treated with lithium.[23] Lithium neuroprotective properties have long been recognized,[45] with some authors postulating a specific effect in countering BD neuroprogression[46]: it is therefore of note that lower NAA levels, possibly indicative of a pathological brain deterioration, could only be identified in individuals not prescribed lithium. In this regard, it is worth stressing that all our analyses were corrected for previous months of lithium treatment in an attempt to control for the likely strong effect of the ion on BD longitudinal course.

Lifetime (hypo)manic episodes also appeared to have a detrimental effect on WM microstructure, with a negative association with FA and a positive association with RD and MD. A decrease in FA and a concomitant increase in RD and MD are usually interpreted as a reduction in myelination of nerve fibers.[47] This result is reminiscent of a recent large ENIGMA study that identified a specific detrimental effect of manic states on brain structure, with a faster rate of cortical thinning in patients with more manic episodes.[12] Concerning WM microstructure, while not explicitly testing the effect of episode number, another large ENIGMA study identified a negative association between illness duration and FA.[16]

The specific relation we identified between lifetime manic episodes and WM microstructure could again reflect a progressive cumulative effect, similar to what was observed for NAA concentrations. NAA is indeed crucial for myelin integrity, as it is synthesized in neurons but catabolized in oligodendrocytes to obtain acetate, necessary for the synthesis of lipids that will contribute to the formation of the myelin sheath.[19,48] Accordingly, in our sample, NAA/Cr levels had a positive widespread association with WM integrity, confirming previous studies[49,50]; further, we identified a significant indirect effect of lifetime manic episodes on WM microstructure through NAA/Cr levels. This might shed some light on the biology underpinning BD neuroprogression.

Albeit still not fully understood, neuronal mitochondria appear to have a crucial role in NAA synthesis[20]; from neurons, NAA is then transported to oligodendrocytes, where it is essential for myelination and myelin repair.[20] Brain NAA concentration could therefore directly reflect mitochondrial function and well-being.[51–53] Mitochondrial dysfunction is believed to be strongly involved in BD pathophysiology,[54] and its role in BD neuroprogression is starting to be postulated.[55] From our results, it is therefore possible to hypothesize a link between cumulative affective episodes, mitochondrial dysfunction, and reduced neuronal NAA synthesis, with subsequent loss of myelin integrity. It must be noted, however, that our analysis also identified a detrimental effect of lifetime manic episodes on WM microstructure

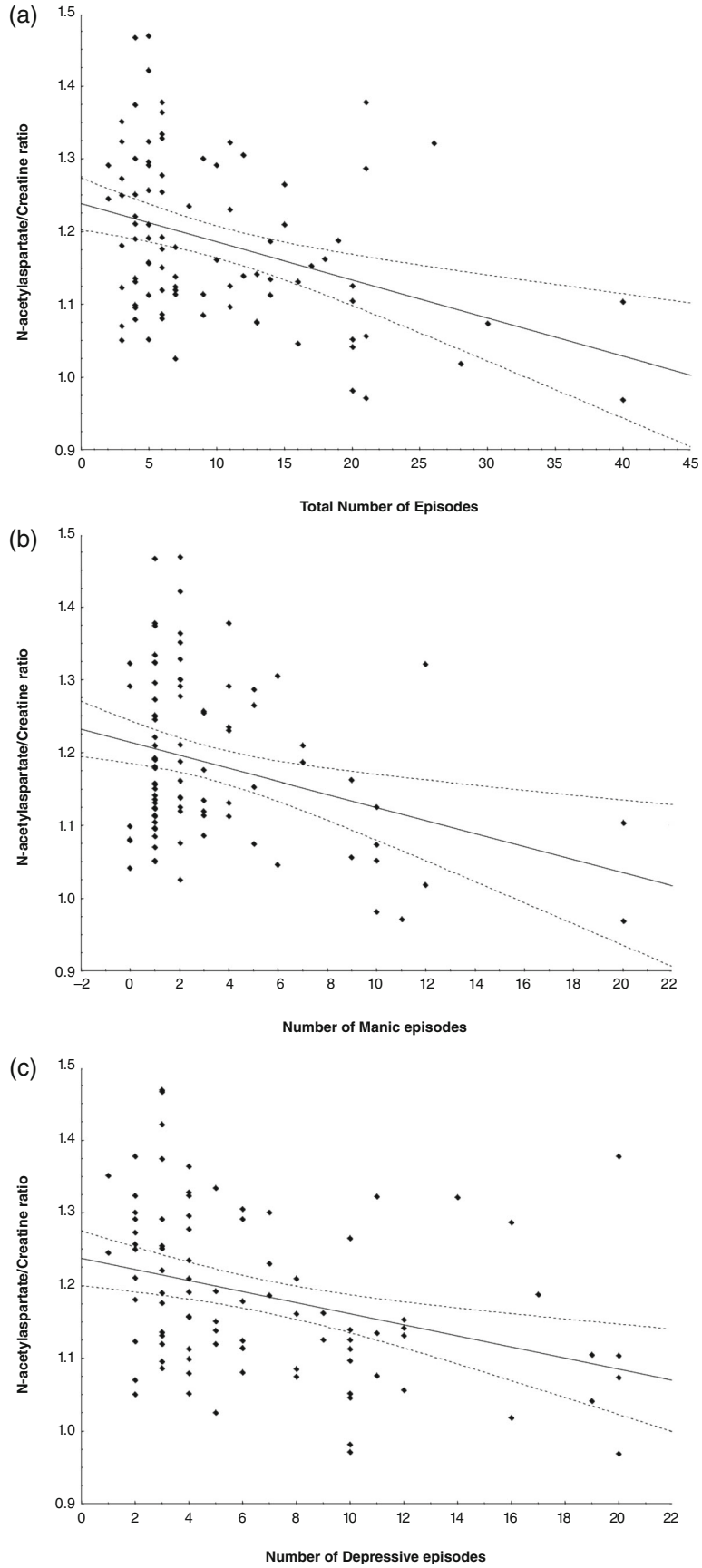

Fig. 1 Associations between NAA/Cr and the number of total mood episodes (a), manic (b), and depressive ones (c).

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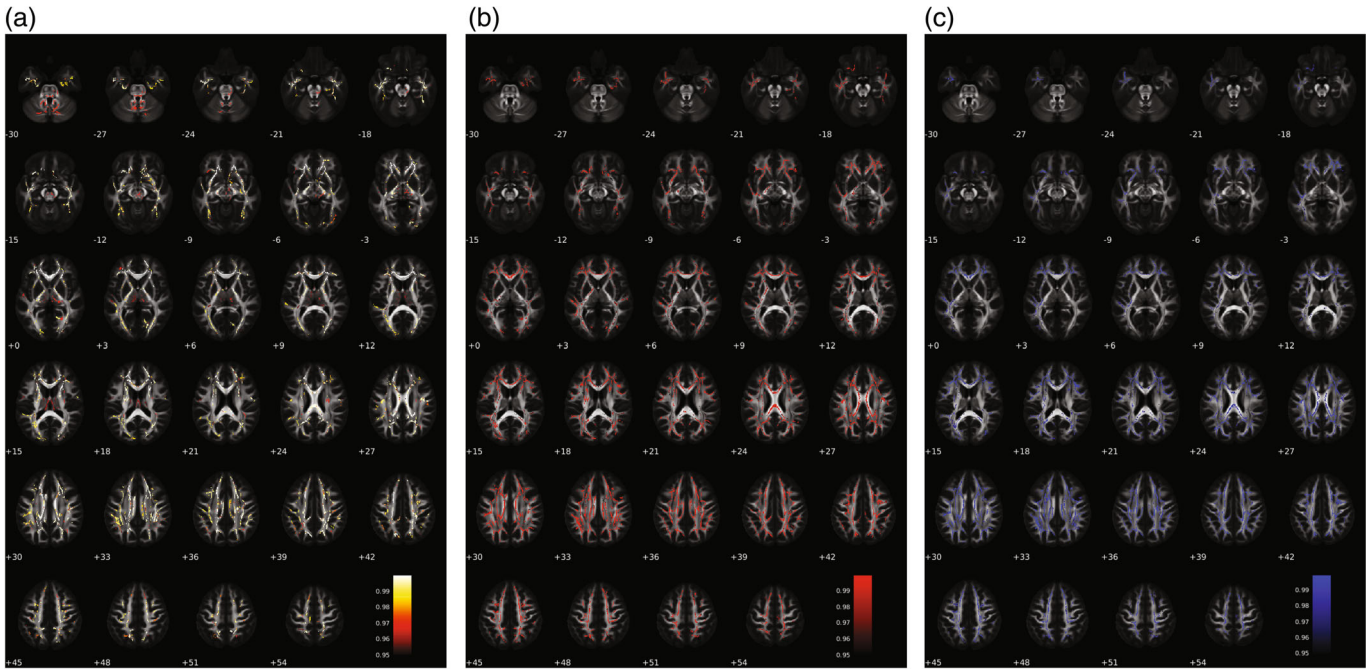

Fig. 2 White matter tracts affected by NAA/Cr ratio. (a) Fractional anisotropy; (b) radial diffusivity; (c) mean diffusivity.

independent from NAA concentrations, suggesting that our hypothesis can account only for a portion of the observed association.

We found the SCZ PRS to significantly moderate the relation between duration of illness and NAA, with significant negative associations between the two only at high PRS values. In the moderated mediation analysis, significant indirect effects of duration of illness on WM microstructure through NAA/Cr emerged once again only for high PRSs.

Large population-based studies have repeatedly linked elevated SCZ-PRS with more 'schizophrenia-like' bipolar phenotypes, such as persistent psychosis, reduced inter-episode remission, and lower psychosocial functioning.[29] Similar trends have been reported for episode polarity and treatment resistance.[27,56] Our data extend this evidence by suggesting that SCZ-PRS also stratifies BD with respect to objective neurobiological markers of illness progression, possibly accounting, at least in part, for the observed neuroprogressive course of a portion of BD patients.[57]

SCZ and BD exhibit extensive genetic overlap,[58–60] and mitochondrial dysfunction is emerging as a pivotal pathogenetic mechanism also in the case of SCZ and its deteriorating course.[61,62] In the hypothesis that brain NAA concentrations are at least in part reflective of neuronal mitochondrial activity,[51–53] our results might suggest the presence of a progressive mitochondrial dysfunction, unrelated to the cumulative effect of affective episodes, in the portion of bipolar patients with higher SCZ genetic risk. This process could be to some extent related to the longitudinal changes seen in the progressive forms of SCZ.[63] Indeed, partially corroborating our results, NAA levels have been found to be reduced only in chronic and not in first episode SCZ[64] and to be negatively associated with illness duration.[65]

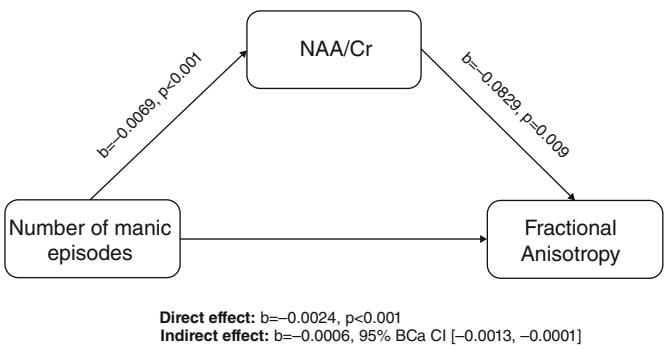

Fig. 3 Mediation analysis: effect of the number of manic episodes on fractional anisotropy through NAA/Cr ratio.

Finally, a moderating effect of SCZ-PRS also emerged in the relation between NAA/Cr and FA, with weaker positive associations between the two at increasing PRS values. This finding shifts the focus from neuronal NAA production to oligodendrocytes' uptake and use in myelin formation. While to our knowledge, no study explicitly tested the role of SCZ genetic risk in affecting the relation between NAA and WM microstructure, oligodendrocyte dysfunction and dysregulation in myelination are established features of SCZ and its genetic architecture.[66–68] Although not explicitly the focus of our investigation, our data seem to suggest that SCZ genetic load is also associated with alterations of myelin homeostasis in bipolar patients, with a reduced efficiency in utilizing NAA to preserve myelin integrity.

This study is among the largest investigating brain spectroscopic measures in BD, and to our knowledge the first to test genetic vulnerability impact on the relation between illness course and brain metabolites. It presents however several limitations: The main one is its cross-sectional nature, which hampers our ability to draw causal inferences from the associations we identified—this is particularly relevant when hypothesizing a progressive course of BD, which would be better investigated with a longitudinal study design.

While our sample size is in line with neuroimaging studies, it is smaller than what is usually employed in genetic investigations: This might limit the reliability of our results, especially for interaction effects that are particularly sensitive to sampling variability and may be inflated in smaller samples. Therefore, although the results are consistent with our theoretical framework, replication in larger samples would help to further substantiate these findings.

Our study was performed in a single center, thus limiting generalizability; the lack of a control group does not allow us to estimate the extent to which some of our results (e.g., the association between

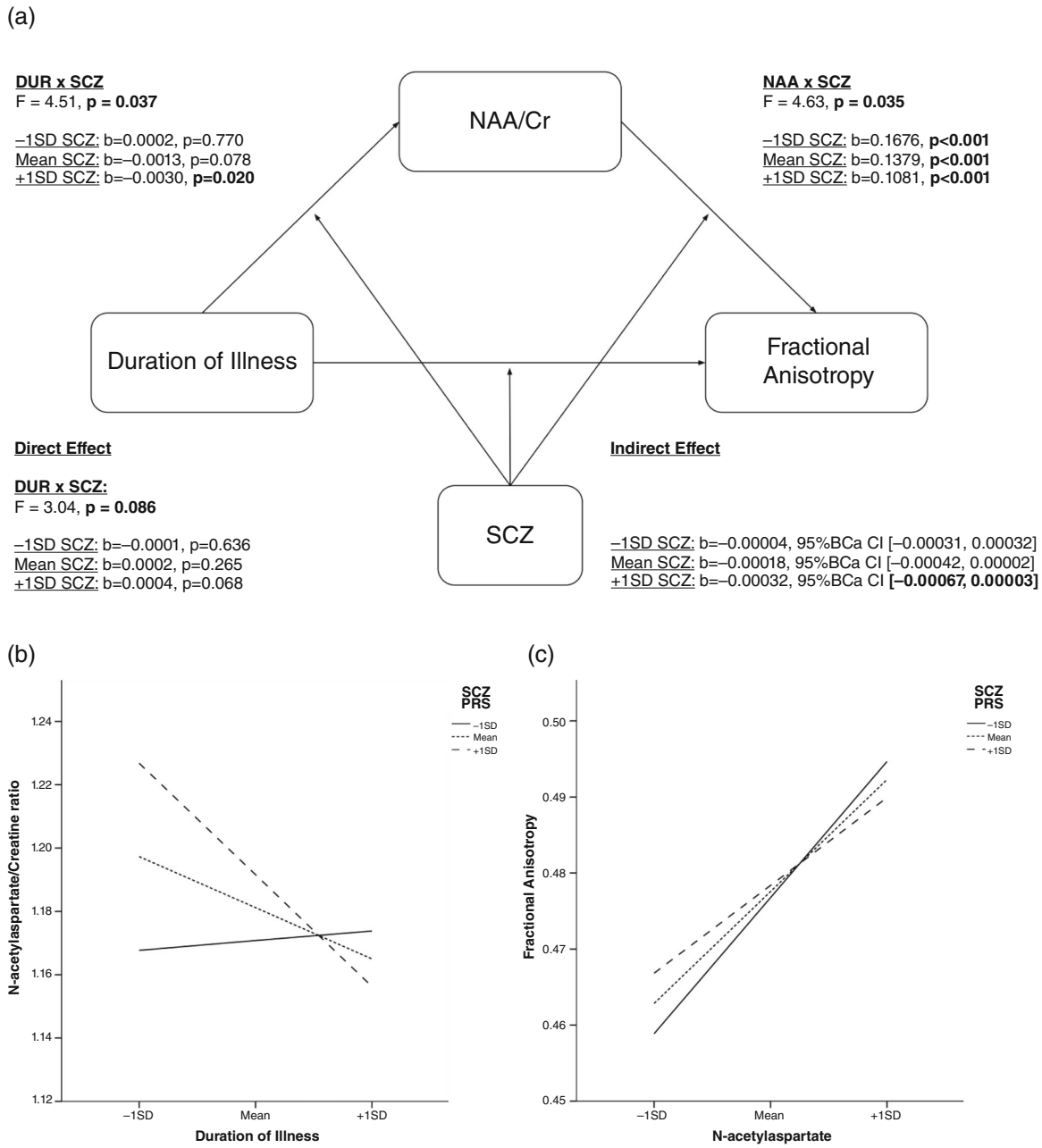

Fig. 4 (a) Moderated mediation model: indirect effects of illness duration on fractional anisotropy through NAA/Cr, moderated by Schizophrenia polygenic score. (b) Relation between illness duration and NAA/Cr moderated by SCZ-PRS. (c) Relation between NAA/Cr and fractional anisotropy moderated by SCZ-PRS.

NAA and WM) are specific to BD. Genetic data were available only in a subset of participants.

Given the real-world nature of our study, many confounding factors that we did not take into account could have affected our results, including differences between BD type 1 or 2 and the presence of common medical comorbidities or risk factors; concerning drug treatment, all the participants in our investigation were prescribed a wide variety of psychotropic drugs: While we controlled all our analyses for lifetime months of lithium treatment, we could not account for the whole variety of prescribed pharmacotherapies.

Despite these limitations, our study significantly adds to the molecular understanding of brain changes associated with BD clinical course: The association we identified between lifetime manic episodes, brain NAA levels, and WM integrity could highlight a potential pathophysiological mechanism involved in BD neuroprogression; the effect of SCZ genetic liability might contribute to the understanding of this complex process, identifying subgroups of BD patients with a partially shared biology with SCZ, further stressing the strong biological commonalities among psychiatric disorders and the need to refine our current nosological classification.

Author contributions

M.P., A.G., F.B., and S.P. conceptualized the study. C.L. and S.S. performed genetic material extraction and genotyping. B.B. and M.C. performed MRI spectroscopy and DTI data preprocessing. L.F.U. and M.P. performed genetic quality check, imputation and PRS calculation. M.P., L.R., A.G., and B.B. performed neuroimaging and genetic analyses. M.P. and A.G. wrote the original draft of the paper. R.Z., C.C., F.B., A.S., and S.P. all contributed to the revised version of the manuscript. F.B. and S.P. supervised all

steps of data acquisition, preprocessing, statistical analyses and manuscript drafting.

Disclosure statement

All authors declare that there are no conflicts of interest in relation to the subject of this study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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