ANALYSES OF PERIPHERAL BLOOD DENDRITIC CELLS AND MAGNETIC RESONANCE SPECTROSCOPY SUPPORT DYSFUNCTIONAL NEURO-IMMUNE CROSS TALK IN TOURETTE'S SYNDROME

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INTRODUCTION

Tourette syndrome (TS) is a childhood-onset neurodevelopmental disorder characterized by the coexistence of motor and phonic tics. Approximately 90% of TS patients manifest one or more neurodevelopmental and psychiatric comorbidities, in particular attention deficit/hyperactivity disorder (ADHD), obsessive–compulsive disorder (OCD), anxiety and depression[1]. Evidence supports a dysfunctional neuro-immune cross talk in TS and other neurodevelopmental disorders, such as autism and ADHD, which might contribute to abnormalities in the trajectory of development of cortico-basal ganglia and cortico-cortical connections[2,3]. Microglia, the brain-resident mononuclear phagocytic cells, are thought to play a central role in these interactions. Transcriptomic studies revealed an association between microglial hyperactivation and dysfunction and TS[4,5]. Population-based epidemiologic and genome-wide association studies converge in demonstrating co-occurrence and genetic correlation of TS with highly prevalent autoimmune and allergic conditions[6,7]. At a systemic level, patients with TS have shown dys- or hyper-regulated cell-mediated pro-inflammatory responses suggestive of an 'inflammatory' state, as well as altered distribution of some immune regulatory cell types (e.g. T-regulatory lymphocytes) consistent with predisposition to autoimmunity. Finally, active immunization by direct injection of cytokines or patients' serum anti-neuronal antibodies replicated TS-like behaviours in mice[8,9,10].

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Circulating peripheral blood dendritic cells (DC) constitute a critical link between innate and adaptive immunity. They represent a heterogeneous population of professional antigen-presenting cells comprising three major DC subsets: plasmacytoid (PDC), myeloid type 1 (MDC1), and myeloid type 2 (MDC2)[11]. DC are implicated in the pathogenesis of numerous autoimmune conditions including multiple sclerosis (MS), psoriasis, type-1 diabetes and systemic lupus erythematosus[12]. An increased frequency of MDC1 was found in autistic children compared to typically developing controls, supporting DC-related immune dysfunction[13,14]. Circulating peripheral blood DC subsets and their relationship to neuroinflammation remain under-investigated in other neurodevelopmental disorders, including TS.

Amongst several applications to the study of brain metabolism, proton magnetic resonance spectroscopy (1H-MRS) has the potential to provide insight into *in vivo* neuroinflammatory changes through the quantification of different metabolites[15], as markers of neuronal or glial damage in selected brain regions[16]. For example, N-acetylaspartate changes were previously described in patients with neurological manifestations of lupus erythematosus[17,18], while choline and lactate compound abnormalities were linked to active inflammatory demyelination and neuronal injury in MS[19,20].

In this study, we first investigated the frequency and distribution of circulating peripheral blood-derived DC in TS patients, comparing them to age- and sex-matched healthy volunteers (HV). Subsequently, we explored relationships between DC subsets and clinical severity of tics and comorbid behavioural symptoms, accounting for the potential influence of exposure to psychotropic medications. Finally, we aimed to investigate the relationship between brain metabolites associated with glial activation/inflammation obtained via quantitative MRS and peripheral blood DC frequency to determine whether this supports the hypothesis of an active crosstalk between central nervous and immune systems in TS. Our primary hypothesis was that TS patients would exhibit an abnormal distribution of the different DC subsets, and that this abnormality would be greater in patients with a greater burden of behavioural comorbidities.

METHODS

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Participants

Patients were recruited from the St. George's University Hospital Tic Disorder and Movement Disorders clinic if they fulfilled DSM-5 diagnostic criteria for TS and had received stable pharmacological treatment for the previous 3 months. HV without neurological diagnoses were enrolled amongst patients' friends or partners. Exclusion criteria were: autoimmune disorders; ongoing acute/chronic infections; chronic obstructive pulmonary disease; malignancies and chronic endocrinological, cardiovascular, pulmonary, liver or kidney diseases; treatment with corticosteroids or immunosuppressant drugs within the previous 12 months. The study was approved by the London-Westminster Research Ethics Committee (project ID 216892).

Clinical assessment and sample collection

All participants were administered the Yale Global Tic Severity Scale (YGTSS)[21], Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)[22], Adult ADHD-Rating Scale (ADRS)[23], Beck Depression Inventory-II (BDI-II)[24] , and Beck Anxiety Inventory (BAI)[25]. The YGTSS, Y-BOCS and ADRS instruments were administered by the same trained neurologist (MS). Comorbid OCD and ADHD were diagnosed using DSM-5 criteria.

The presence of depressive and anxiety symptoms was determined based on BDI-II score >14 and BAI score >8 (the latter indicating the presence of mild, moderate or severe anxiety)[24,25].

After clinical assessment, 10 ml of venous EDTA-anticoagulated blood were collected from each participant for immunological characterization. Samples were transferred to the laboratory and stored at 4°C for ≤3 hours before being processed for immunophenotyping. On the same afternoon, participants underwent MRI scan to obtain 1H-MRS data.

Quantification of circulating peripheral blood DC subsets

DC subsets were identified using the Human Blood DC Enumeration kit (Miltenyi Biotec). As per manufacturer's protocol, fresh peripheral blood samples (300ul of EDTA-blood) were stained with an antibody cocktail containing: antibodies directed against CD19 (CD19-PE-Cy5) for exclusion of B cells, antibodies directed against CD14 (CD14-PE-Cy5) for exclusion of monocytes; and antibodies against BDCA-1 (CD1c-PE), BDCA-2 (CD303-FITC) and BDCA-3 (CD141-APC) to identify MDC1

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(BDCA-1+), MDC2 (BDCA-3+), and PDC (BDCA-2+) **(Figure 1)**. Each sample was stained alongside an isotype control mouse antibody cocktail containing IgG1-FITC, IgG2a-PE, IgG1-APC. Dead cells were excluded using a dead cell discriminator dye (PE-Cy5) **(Figure 1)**. Samples were washed with phosphate buffer saline (0.5% bovine serum albumin) and fixed. Flow cytometry data acquisition were performed within 3-6 hours from collection using a Navios (Beckman Coulter) and a FACSCalibur (BD Biosciences) flow cytometers, subsequently analysed with the FlowJo software (FlowJo, LLC).

1H-MRS data acquisition

1H-MRS data were acquired using a Philips 3T dual Tx Achieva MRI system with a 32-channel head coil. Sagittal 3D T1-weighted (T1w) images were acquired to provide high grey/white matter contrast that depicts brain anatomy and allows accurate MRS voxel placement (acquisition parameters: 1x1x1.5mm resolution, inversion time TI=998ms, TE=3.8ms, TR=7.8ms, flip angle 8 degrees, acquisition time 4.5 minutes). MRS voxel localisation was focused on left putamen (PUT) (voxel size 30x12x10mm) and subcortical frontal white matter (FWM) (voxel size 20x12x12mm) of the right hemisphere. MRS voxel placement was performed always by the same operator, with voxels oriented obliquely to the three image planes to maximise tissue of interest and exclude surrounding tissue, as shown in **Figure 2**. 1H-MRS data were obtained using the single volume Point-RESolved Spectroscopy sequence at short echo time TE=32ms with repetition time TR=2000ms. Metabolite spectra were acquired with 192 averages and a non-water suppressed acquisition of the tissue water acquired with 16 averages. Each acquisition lasted 6.5 minutes. Patients alerted the operator to their own tics during scans, after which lower resolution 3D T1w images (acquisition time 51 seconds) were always acquired after each 1H-MRS acquisition to allow visual assessment of patient's movement, repeating 1H-MRS if deemed necessary. The total MRI scan time was approximately 30 minutes, including repetitions of 1H-MRS acquisitions.

1H-MRS data was analysed using LCModel version 6.31[26] to determine the signal intensities of combined N-acetylaspartate and N-acetylaspartyl glutamate (tNAA), glutamate plus glutamine (Glx), total creatine plus phosphocreatine (tCr), total choline and phosphocholine and glycerophosphocholine (tCho). Results are reported as metabolite concentrations (mM) using the tissue water signal as a

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reference (assumed 41.7M). No corrections for relaxation time effects or tissue partial volumes within the MRS voxel were made.

Data analysis

All data were analysed using IBM SPSS Statistics 23. The normality assumption for all measures was confirmed by Kolmogorov-Smirnov test ($p>0.05$). Frequencies of DC and 1H-MRS metabolite brain levels in TS and HV were compared using two-sample *t*-tests. The level of significance was set at $p<0.05$ (two-tailed).

Relationships between DC subset frequencies, 1H-MRS metabolites and demographic and 1H-MRS quality parameters were first explored with bivariate correlations. As preliminary analysis revealed significant correlations between metabolite concentrations and age and 1H-MRS linewidth (full width half maximum, FWHM), these parameters were used subsequently as covariates in bivariate correlation and a General Linear Model (GLM) analyses.

We subsequently analysed the association of psychiatric comorbidities and drugs with DC subset frequencies and 1H-MRS brain metabolites in TS patients. TS were divided into subgroups with (TS+) and without (TS-) a specific pre-defined psychiatric comorbidity, i.e. ADHD and OCD, or psychiatric symptom domain, i.e. anxiety and depression. For each comorbidity/comorbid symptom domain, ANOVA was used to assess the effect of 'group' (TS+, TS- and HV); where significant, post hoc *t*-tests with Bonferroni correction were used to perform pairwise comparisons between groups (significance level $p<0.05$). Similarly, to explore associations with drug exposure. TS patients were divided into TS with (TS+) and without (TS-) exposure to antipsychotic drugs, and the effect of 'group' was explored with ANOVA. Effects of other medication classes and daily tobacco smoking (according to WHO's Smoking and Tobacco Use Policy definition[27]) were evaluated conducting sensitivity analyses after exclusion of TS patients with each specific drug class. For each class, between-group differences between HV, TS+ and TS- groups were explored using ANOVA with post hoc *t*-test with Bonferroni correction where significant (significance level $p<0.05$). In cases where one group contained fewer than 2 patients, an independent t-test comparing the remaining two groups was performed instead of ANOVA (significance level $p<0.05$).

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Finally, unilinear GLMs were used to test possible explanatory and confounding factors or adjust for covariates where significant correlations were found according to our predefined cut-offs. Statistical significance at GLMs was defined as $p<0.05$.

RESULTS

Eighteen TS patients and 18 HV entered the study. The two groups were similar for demographic characteristics. Scores for ADRS, BAI and BDI were significantly higher in TS patients compared to HV (p=0.003, p=0.001, p=0.014, respectively; **Supplementary Table 1**). Amongst comorbid disorders/symptoms, OCD was present in 8 TS patients, ADHD in 8, anxiety in 12 and depression in 5. Fifteen patients were treated for tics or other behavioural symptoms with the following medications: aripiprazole (n=5), botulinum toxin (n=4), clonidine, pimozide, sulpiride and clonazepam (n=1 each), sertraline (n=2), amitriptyline, clomipramine and atomoxetine (n=1 each); 3 were chronic cannabis users; 3 were daily tobacco smokers and none was on behavioural treatment or had undergone functional brain surgery.

Data from one patient and two HVs were excluded because of staining failure. We did not observe significant between-group differences in frequency of MDC1 (TS, 0.60±0.20%, HV, 0.55±0.18%, p=0.41), MDC2 (TS, 0.049±0.02%, HV, 0.046±0.01%; p=0.52), and PDC (TS, 0.36±0.13%, HV, 0.41±0.16%, p=0.42) subsets. ANOVA comparing DC subset frequencies between TS patients with or without behavioural comorbidities and HV yielded a significant 'group' effect when TS patients were sub-grouped by anxiety symptoms (p=0.025; **Table 1**); *post hoc* analysis showed significantly higher MDC1 frequency in TS+anxiety compared to TS-anxiety (p= 0.01; **Figure 3**). We did not detect any other significant association between other comorbidities and DC subset frequencies **Table 1**). Similarly, we could not identify any significant correlation between severity of tics, ADHD, OCD, depressive and anxiety symptoms, and frequency of DC subsets (**Supplementary Table 2**). Finally, ANOVA comparing TS patient subgroups divided according to current antipsychotic exposure and HV did not show any significant effect of clinical group (**Supplementary Table 3**). Likewise, sensitivity analyses testing the potential impact

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of other drugs on DC subset frequency did not reveal significant associations (**Supplementary Table 3**).

1H-MRS data from TS patients (2 FWM, 3 PUT) and HV (3 FWM, 1 PUT) were excluded after visual assessment of spectrum quality prior to any analysis, due to poor water suppression, excessive baseline roll, artefactual peaks, poor peak resolution or low signal to noise. LCModel assessment of quality of accepted data gave mean and standard deviation of the water FWHM and metabolite signal-to-noise ratio of 0.036±0.005 ppm and 14.8±2.5 respectively in FWM (n=31), and 0.069±0.02 ppm, 14±1.7 respectively in PUT (n=32), without significant differences between TS patients and HV.

Metabolite concentrations change with age[28] and age-related changes in iron deposition in the basal ganglia[29] may also change water relaxation times[28], thus affecting metabolite estimates. PDC are also known to decrease with age[30]. In a preliminary correlation analysis, we observed significant correlations ($0.05>p>0.011$) between several metabolites, age and FWHM in the putamen of HVs and patients, as well as an inverse correlation of PDC with age (r=-0.400, p=0.021). Hence, FWHM and age were used as covariates to assess correlations between metabolite concentrations and cell counts.

We found a strong negative correlation between tCr and MDC1 subset in the FWM of TS patients (r=-0.784, p=0.0015), which survived a Bonferroni-corrected p value of 0.0021 for 24 comparisons (four metabolites, three cell types and two regions), but not in PUT (r=-0.444, p=0.148) **(Table 2)**. Other correlations significant at $p<0.05$ (not Bonferroni-corrected) were: tNAA with PDC (r=-0.588, p=0.035) in FWM of TS, and Glx with PDC (r=0.651, p=0.022) in PUT of TS patients. No significant correlations were present for both FWM and PUT in HV **(Table 2).** The correlation of tCr with MDC1 in FWM of TS patients was also highly significant without covariates (r=-0.797, $p<0.001$; **Figure 4a**). Although not significant, there was a trend for a tCr decrease with MDC1 in putamen, which closely matches that of the correlation in FWM (**Figure 4a**); this correlation was not found in HV (**Figure 4b**). A general linear model investigated the relationship between tCr and MDC1 including both putamen and FWM data, with age and FWHM as covariates. tCr

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correlated to MDC1 across both anatomical regions with F=12.61, p=0.002, with also a significant age effect and highly significant effect size for location (**Supplementary Table 4**). Finally, sensitivity analyses testing the potential impact of drug classes on tested brain metabolites either in FWM and putamen did not show significant associations (**Supplementary Tables 5A and 5B**).

DISCUSSION

To our knowledge, our study is the first to investigate the distribution of circulating DC subsets in TS and to explore its relationship with the comorbidity profile of TS. In contrast to results reported in autism[13,14], we did not observe differences in the frequency of circulating DC subsets between TS patients and age-matched HV. Whereas the frequency of DC subsets did not correlate with tic severity, we detected an increase in MDC1 frequency in TS patients manifesting anxiety symptoms (mild, moderate or severe). Moreover, our analysis of a possible association between DC subset frequencies and metabolite levels within fronto-subcortical network regions showed a strong correlation between MDC1 frequency and tCr levels in the FWM of TS patients, but not in HV. This observation was independent of anxiety and supports a possible relationship between systemic immunoregulatory mechanisms and brain metabolism in adults with TS.

The lack of correlation between DC subset frequency and clinical severity of tics and comorbid diagnoses or symptoms (i.e. OCD and anxiety) does not support a direct influence of immune mechanisms regulated by, or influencing the activity of, DC on the frequency and intensity of the abnormal behaviours typical of the TS spectrum.

Our findings support, rather, that MDC1 frequency could be associated with the presence of anxiety symptoms in TS patients. Longitudinal observations would add more clarity on whether MDC1-regulated immune mechanisms promote the development of anxiety symptoms, are accelerated by stress responses and anxiety, or represent epiphenomena that lack a direct mechanistic relationship with behavioural features.

The relationship between stress responses, anxiety symptoms and systemic immune regulation in chronic tic disorders, and the related contribution of autonomic

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and neuroendocrine signalling mechanisms[31,32] remain heavily under-investigated. They may involve peripheral effects, including abnormal interleukin production that may drive naïve self-reactive T cells to react against CNS tissue, or failure to generate/maintain T-cell tolerance via negative selection in the thymus. The increased prevalence of generalized anxiety disorder in the TS population is well recognized[33], as well as the increased level of circulating cortisol and pro-inflammatory cytokines in individuals with generalized or other anxiety disorders[34,35]. Mild anxiety symptoms have also been associated with altered gene expression patterns of innate and adaptive immune responses[36]. It has been shown that the increase in circulating corticosteroid levels during stressful events can precipitate a dysfunction of cell-mediated immune processes also by disrupting DC maturation[37] and antigen presentation functions and, therefore, their ability to generate an effective T-cytotoxic response[38,39]. To our knowledge, however, the effect of corticosteroids on peripheral blood circulating DC subsets has never been investigated in the context of neuropsychiatric or neurodevelopmental disorders. Among DC subsets, MDC1 have a specific capability to present antigens via MHC class II to activate naïve $CD4^+$ T cells, and to promote T helper 1 responses[11]. As previously demonstrated in animal models of MS, clinical manifestations of CNS autoimmunity are preceded by a phase of microglia expansion and myeloid DC peripheral proliferation[40,41]. The peripheral increase of MDC1 in TS patients with increased anxiety symptoms may reflect a pro-inflammatory state possibly facilitating an altered neuro-immune crosstalk.

To date, 1H-MRS studies of TS highlighted neurochemical changes associated with this diagnosis and/or with tic severity with some inter-study heterogeneity[42-44]. In line with our findings, previous reports demonstrated a reduction of tCr in putamen, right frontal cortex and thalamus[43,45]. The central role played by creatine and creatine kinase/phosphocreatine in high metabolism cells, such as brain and muscle, by regenerating adenosine triphosphate from adenosine diphosphate is widely recognized[46,47]. Genetically determined deficits of creatine phenotypically present with severe neurological symptoms from young age[48]. The strong inverse correlation between brain tCr levels and MDC1 subset frequency in our TS patients lends support to a potential association between metabolic changes of brain regions that are directly involved in the generation and control of pathological behaviours in TS and a systemic inflammatory state. Alternatively, this

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correlation could suggest a direct influence on immune regulatory mechanisms at a systemic level[49,50] exerted by a generalized alteration of creatine metabolism, expressed here by lower concentrations of tCr in different brain regions. In particular, creatine kinase B (CK-BB; brain type) has been reported as a regulator of T cell development and activation through the control of T-cell receptor (TCR) signalling during negative selection in the thymus[51], a key mechanism for self-antigen tolerance and the pathogenesis of autoimmune disease. If altered creatine metabolism influences CK isoform activity, a potential effect of this could be a dysfunction in the regulatory effect of CK-BB upon TCR signalling in T cells, contributing to their dysregulation, promotion of an inflammatory state, and predisposition to autoreactive immune processes, particularly in TS patients with co-existing anxiety. A more focused exploration of creatine metabolism in TS and related disorders is needed to appraise this alternative interpretation. Furthermore, the observed correlation between brain tCr concentrations and peripheral blood MDC1 frequency was not influenced significantly by anxiety and depressive symptoms or behavioural comorbidities, suggesting that this link is not directly related to concomitant emotional or behavioural abnormalities in TS patients.

We acknowledge several limitations of our study. First, our sample may not be representative of the general TS population, as it involves a subgroup of patients whose tics persisted in adulthood. Second, to avoid skewing our sample towards milder forms of disease as in previous neuroimaging studies on TS[41,50,51], we included patients on stable but disparate pharmacological treatments. Although our sensitivity analyses did not detect any major influence of drugs on outcomes of interest, we recognize that analyses might have missed smaller effects of drug exposure on DC frequencies and brain metabolite spectra. Third, the presence of anxiety symptoms in our TS patients was determined only on the basis of the BAI score, and the majority of them scored in the range of mild-to-moderate symptoms. The small TS patient group size precluded sufficient statistical power to assess associations between MDC1 frequency and severity of anxiety symptoms or a clinical diagnosis of comorbid anxiety disorder. Fourth, other factors might have influenced the brain metabolites explored in our study. Levels of Glu and Gln may be influenced by nicotine[52] and sleep patterns[53,54]. Similarly, tNAA levels may be affected by nicotine use[52], lactate may increase following caffeine ingestion[55] and choline and Glx levels exhibit diurnal variations[54,56]. A region-dependent reduction in

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tCr was reported in middle-aged smokers compared to non-smokers[52]. Although controversial[57,58], a sex-dependent variation of all metabolites was previously suggested for specific brain regions[59,60]. Yet, our sensitivity analyses did not detect a significant confounding effect of tobacco smoking on tCr-MDC1 observed relationship and an effect of daily variation is unlikely as MRIs were all performed in the afternoon. We nevertheless acknowledge that undetected effects of caffeine, poor sleep quality and lack of strict matching by sex might have increased the variability of metabolite levels in both groups, potentially obscuring subtle inter-group differences. Finally, the limited availability of cerebrospinal fluid specimens from this patient population and lack of access to *in vivo* molecular imaging markers of neuroinflammation did not allow us to correlate DC frequencies to neuroinflammatory processes beyond the information that could be provided by metabolic spectra.

In conclusion, here we report an increase of the MDC1 subset of DC in adults with TS and concurrent anxiety symptoms (mild, moderate or severe), which might be associated with a systemic inflammatory state described in patients with this neurodevelopmental disorder. Moreover, the strong correlation between this DC subset and decreased tCr in the FWM of TS patients could originate from immune dysregulation predisposing/contributing to inflammation, suggesting tCr as a marker of inflammatory changes in TS. Finally, our results support the importance of exploring the influence of the whole array of behavioral symptoms, beyond the primary diagnostic feature when investigating immunological and other regulatory mechanisms in complex neurodevelopmental disorders.

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Data availability statement. The data that support the findings of this study are available from the corresponding author, DM, upon reasonable request.

FIGURE LEGENDS

Figure 1. Quantification of circulating dendritic cell subsets. The frequency of circulating dendritic cell subsets was determined in fresh peripheral blood samples from healthy volunteers (n=16) and patients with Tourette syndrome (TS, n=17) by

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flow cytometry (detailed in Methods). Illustrative dot plots show the gating strategy: forward scatter (FSC) and side scatter (SSC) parameters were used to create a gate (P1) that excluded debris and platelets. Next, SSC and CD19/CD14/dead cell discriminator (cocktail-PE-Cy5) parameters were used to generate a gate (P2) that excluded B cells, monocytes, granulocytes and dead cells. Then, expression of BDCA-1 (CD1c), BDCA-2 (CD303) and BDCA-3 (CD141) was used to identify myeloid dendritic cells type 1 (MDC1), myeloid dendritic cells type 2 (MDC2) and plasmacytoid dendritic cells (PDC). Dashed rectangular gates display staining with isotype control (Ctrl) antibodies (detailed in Methods) **A.** Healthy volunteer. **B.** Tourette's syndrome patient

Figure 2. Voxel location and LCModel fit to the 1H MRS data in a Tourette's patient. **A)** Frontal white matter; **B)** Putamen. Voxel sizes were 20 x 12 x 12 mm for frontal white matter and 30 x12 x 10 mm for putamen. Voxels were obliquely positioned on the three orthogonal image planes to maximise the tissue of interest within each voxel. Yellow boxes indicate the localization for tNAA, and the white box that for the water resonance for the metabolite acquisition. The tissue water reference signal was obtained from the same region as that of the tNAA signal. Labelled metabolite peaks are: total NAA (tNAA); glutamate plus glutamine (Glx); total creatines (tCr); total cholines (tCho). In the spectra, the red line indicates the LCModel fit to the raw data, the lower line indicates the baseline and the upper plot the residual signal.

Figure 3. Frequency of circulating dendritic cell subsets in patients with Tourette's syndrome. The frequency of circulating dendritic cell subsets was determined in healthy volunteers (n=16) and patients with Tourette's syndrome (TS, n=17) by flow cytometry (detailed in Methods). **A.** Graphs display the percentage of MDC1, MDC2 and PDC dendritic cell subsets in the two study groups (horizontal bars, mean). No significant differences were identified (unpaired two-tailed Student's t test) **B**. Graphs display the frequency of the three dendritic cell subsets in healthy volunteers (n=16), patients with TS and anxiety (TS-Anxiety, n=11), and patients with TS without anxiety (TS no Anxiety, n=6); (horizontal bars, mean). *p=0.01. MDC1,

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myeloid dendritic cells type 1; MDC2, myeloid dendritic cells type 2; PDC, plasmacytoid dendritic cells.

Figure 4A and 4B. Scatter plot of MDC1 and total Creatine (Cr+PCr) correlation by location. A) Tourette's syndrome patients and B) Healthy Volunteers.

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Table 1. ANOVA sub-group analysis of DC frequencies between groups based on psychiatric comorbidities.

		Anxiety		ADHD		OCD		Depression	
		Mean ± SD	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**
% of MDC1	**TS +**	0.69±0.17	11	0.67±0.21	7	0.62±0.23	7	0.76±0.21	5
	TS-	0.44±0.17	6	0.56±0.20	10	0.60±0.20	10	0.54±0.17	12
	HV	0.55±0.18	16	0.55±0.18	16	0.55±0.18	16	0.55±0.18	16
	df	32		32		32		32	
	p	**0.025***		0.36		0.702		0.072	
% of MDC2	**TS +**	0.05±0.02	11	0.05±0.02	7	0.05±0.02	7	0.05±0.01	5
	TS-	0.05±0.02	6	0.05±0.02	10	0.05±0.02	10	0.05±0.02	12
	HV	0.05±0.01	16	0.05±0.01	16	0.05±0.01	16	0.05±0.01	16
	df	32		32		32		32	
	p	0.457		0.733		0.816		0.576	
% of PDC	**TS +**	0.39±0.15	11	0.42±0.14	7	0.37±0.14	7	0.38±0.11	5
	TS-	0.32±0.08	6	0.33±0.11	10	0.36±0.13	10	0.36±0.14	12
	HV	0.41±0.16	16	0.41±0.16	16	0.41±0.16	16	0.41±0.16	16
	df	32		32		32		32	
	p	0.519		0.295		0.716		0.681	

Abbreviations: TS + denotes Tourette's syndrome patients with comorbidity X; TS - denotes Tourette's syndrome patients without comorbidity X; HV, Healthy Volunteers. A= Anxiety, ADHD, Attention Deficit and Hyperactivity Disorder. OCD, Obsessive-Compulsive Disorder. Depression.

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Table 2. Correlations between DC subsets and MRS metabolites in FWM and PUT in TS and HV.

			MRS METABOLITES									
			FRONTAL WHITE MATTER					PUTAMEN				
			N	tCho	tCr	NAA	Glx	N	tCho	tCr	NAA	Glx
TOURETTES	MDC1	Pearson Correlation	15	0.273	**-0.784****	-0.429	-0.202	14	0.085	-0.444	-0.240	0.425
		Sig. (2-tailed)		0.367	**0.002**	0.144	0.507		0.794	0.148	0.453	0.168
	MDC2	Pearson Correlation	15	0.173	-0.124	0.053	0.076	14	-0.100	-0.206	-0.008	-0.076
		Sig. (2-tailed)		0.572	0.686	0.864	0.805		0.756	0.520	0.981	0.814
	PDC	Pearson Correlation	15	0.119	-0.307	**-0.588***	-0.344	14	-0.288	-0.374	-0.262	**0.651***
		Sig. (2-tailed)		0.699	0.307	**0.035**	0.250		0.364	0.231	0.411	**0.022**
VOLUNTEERS	MDC1	Pearson Correlation	13	0.531	0.090	0.212	-0.562	15	0.252	0.016	0.379	0.144
		Sig. (2-tailed)		0.093	0.792	0.532	0.072		0.406	0.958	0.201	0.639
	MDC2	Pearson Correlation	13	0.264	-0.179	0.377	-0.429	15	0.248	-0.026	0.347	0.224
		Sig. (2-tailed)		0.433	0.598	0.253	0.188		0.414	0.933	0.246	0.462
	PDC	Pearson Correlation	13	-0.219	0.089	-0.148	0.377	15	-0.255	-0.229	-0.550	-0.830
		Sig. (2-tailed)		0.517	0.795	0.665	0.253		0.401	0.452	0.858	0.788

tCho= total Choline; tCr= total creatine; NAA= N-acetylaspartate; Glx= glutamate +glutamine; *correlation is significant at the 0.05 level; **correlation is significant at the 0.0021 level (corrected by multiple comparison factor); covariates: age and full-width half maximum (FWHM).

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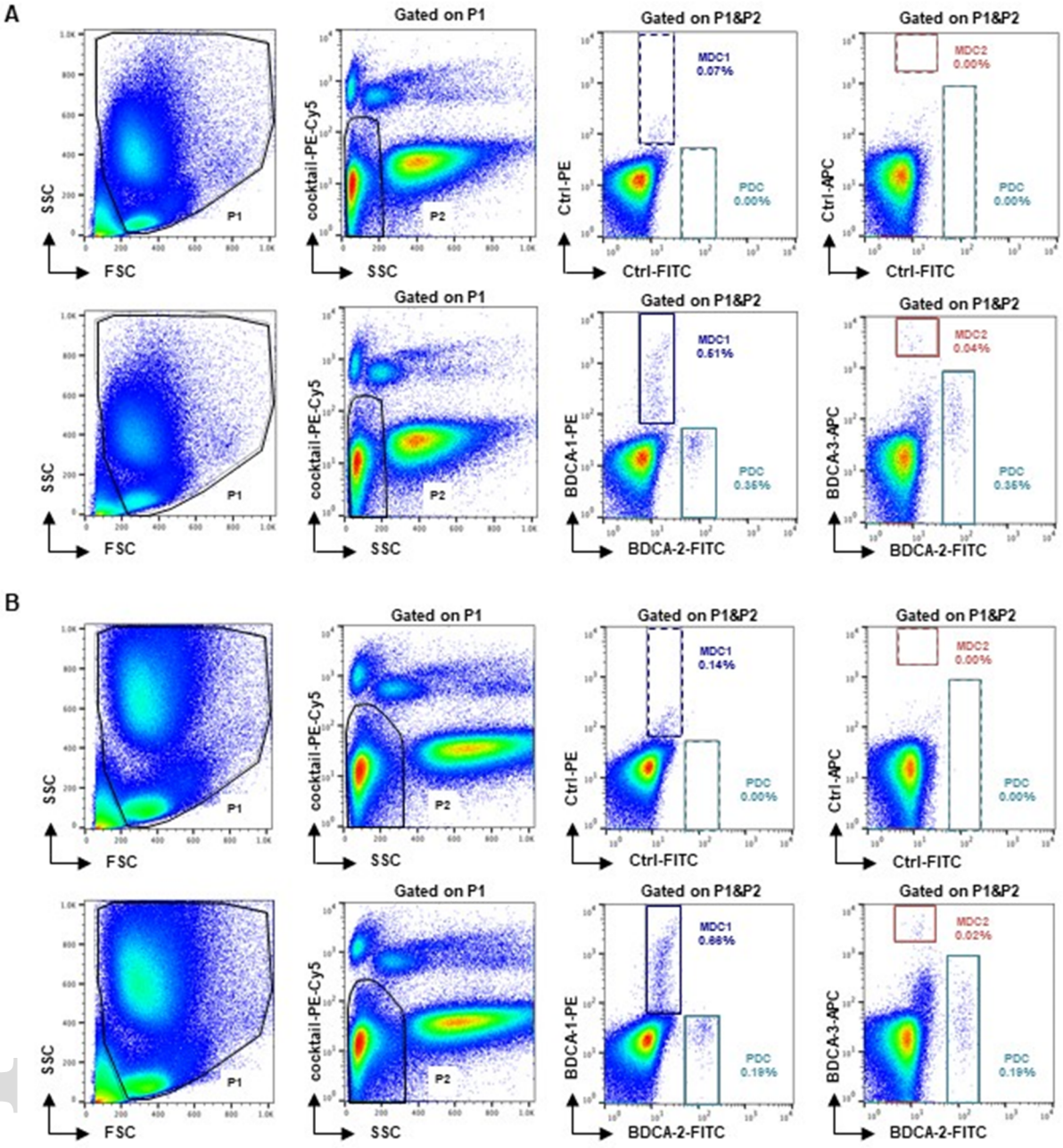

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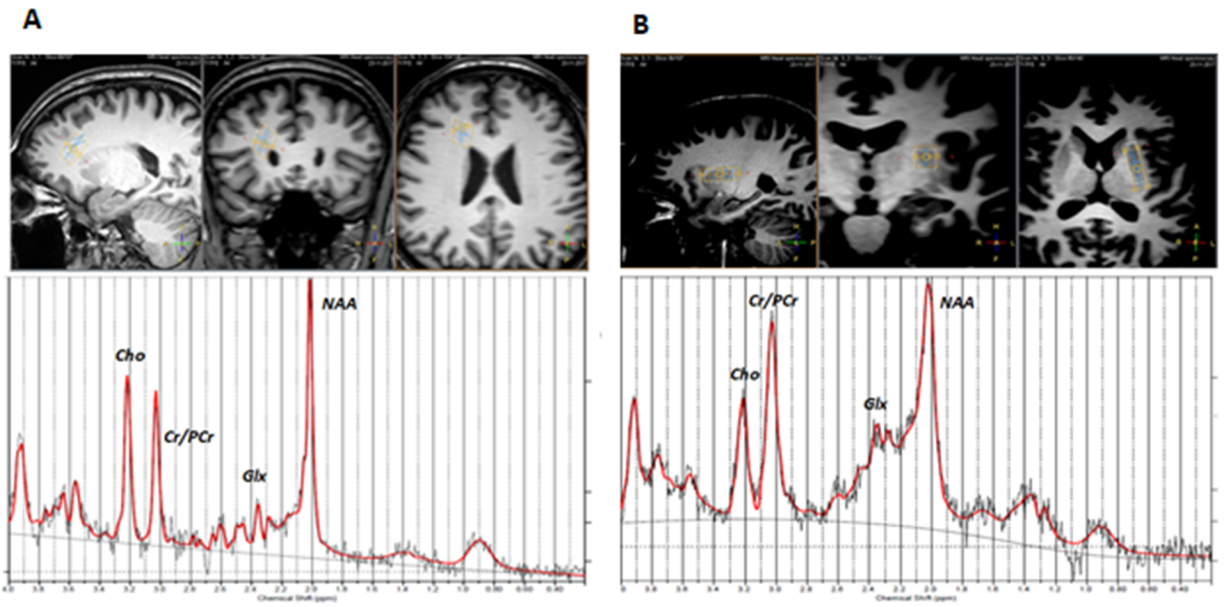

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Figure 3.

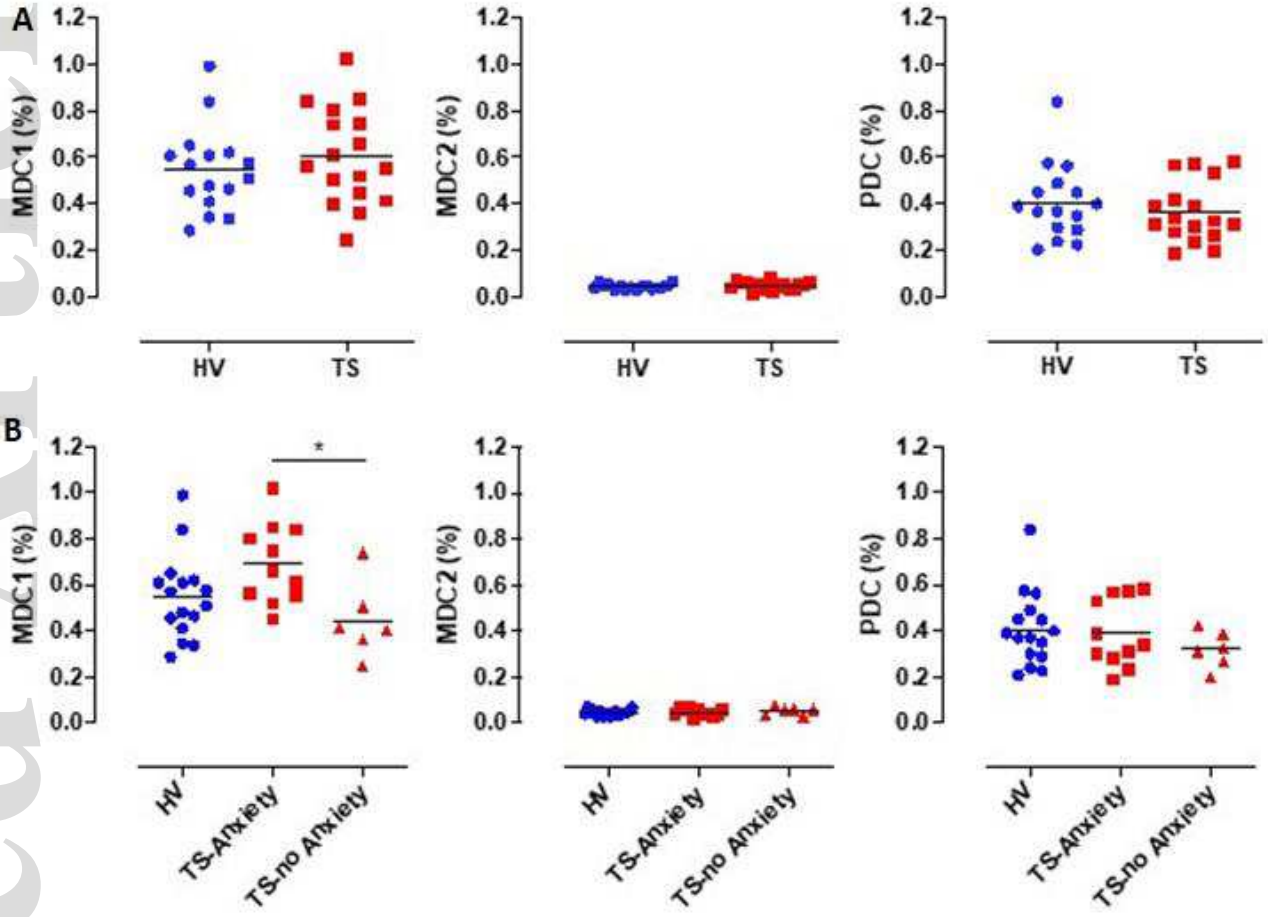

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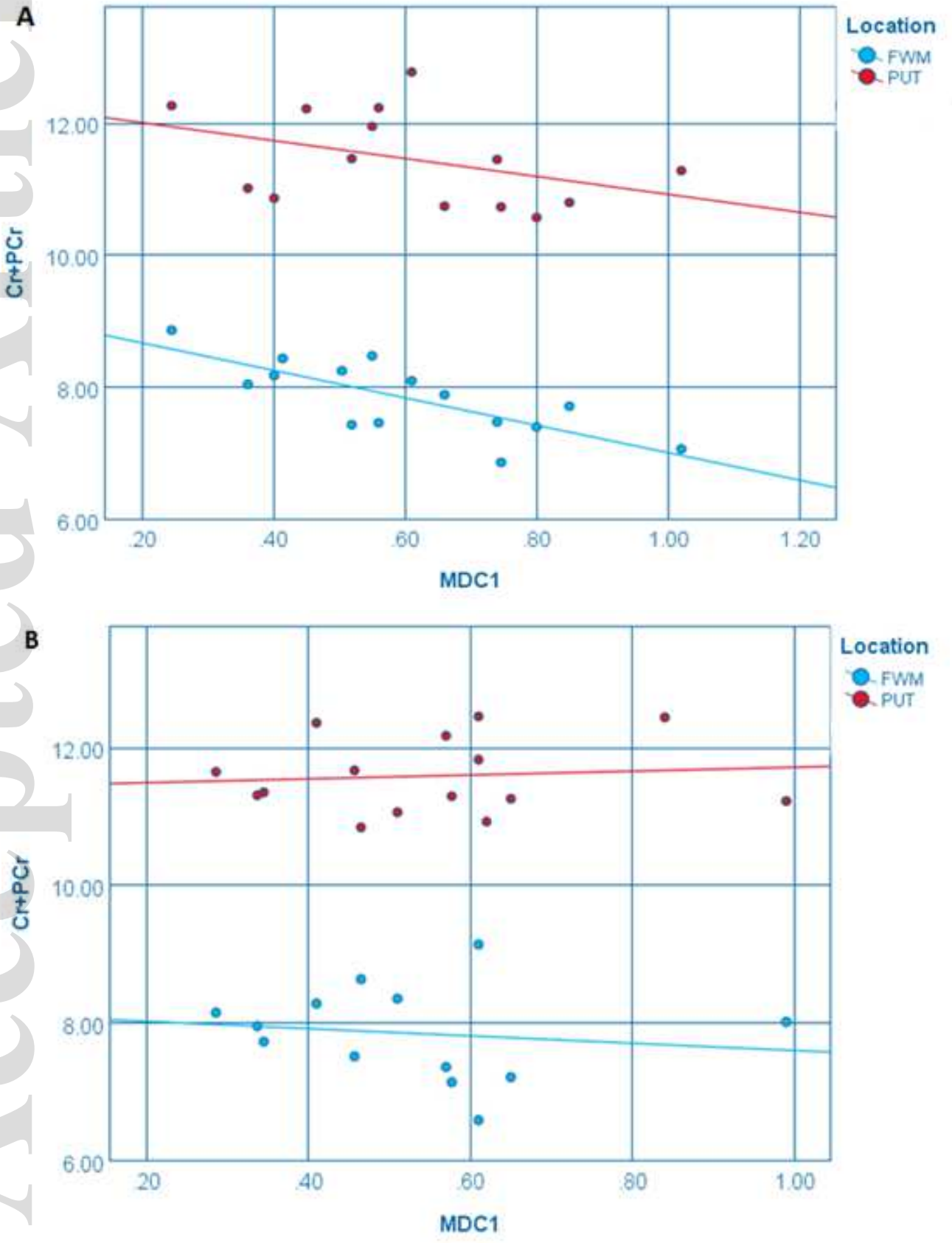
Figure 4A and 4B.



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