CHOROID PLEXUS ENLARGEMENT IN MULTIPLE SCLEROSIS: NORMATIVE TRAJECTORIES, CLINICAL CORRELATES, AND GENETIC ASSOCIATIONS

P057

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INTRODUCTION and PURPOSE

The choroid plexus (ChP), a central component of the blood–CSF barrier, plays a crucial role in CSF production and immune cell trafficking, thereby contributing to CNS homeostasis and immune surveillance [1]. Recent studies have shown that ChP volume increases with age and is enlarged in several neuroinflammatory and neurodegenerative disorders [1]. In multiple sclerosis (MS), ChP enlargement has been observed since the earliest disease stages and is associated with inflammatory activity, white matter (WM) lesion burden, brain atrophy, and clinical features such as cognitive impairment and fatigue [2]. However, the normative trajectory of ChP volume across the healthy adult lifespan and the genetic and structural determinants underlying ChP abnormalities in MS remain incompletely understood.

This study aimed to model normative ChP volume trajectories using a large cohort of healthy controls (HC) and to quantify ChP enlargement in MS through z-scores adjusted for demographic and brain morphometric factors. We further explored associations between ChP enlargement, clinical characteristics, MRI measures of structural brain damage, and genetic susceptibility.

METHODS

- **Subjects.** This retrospective study included 727 MS patients (relapsing-remitting [RR]=477, secondary progressive [SP]=160, primary progressive [PP]=90) and 461 HC, aged 18–70 years.
- **Neurological evaluation.** Disease duration, Expanded Disability Status Scale (EDSS), clinical phenotype, and current disease-modifying therapy (DMT).
- **Brain MRI acquisition and MRI analysis (3.0 Tesla scanners).**
  - Dual-echo (DE) turbo spin echo sequence (Protocol 1) or 3D fluid-attenuated inversion recovery (FLAIR) (Protocol II): quantification of T2-hyperintense white matter (WM) lesion volume (LV) using Jim 8.0, Xinapse Systems Ltd, Colchester, UK and an in-house implemented method.
  - 3D T1-weighted fast field echo (Scanner 1) or 3D T1-weighted turbo field echo (Scanner 2): after lesion filling, quantification of normalized brain (NBV), cortical (NCV), thalamic (NTV), caudate (NCaV), and lateral ventricle volumes (NLVV) using FSL SIENAx2.
- **ChP segmentation.** Performed using ASCHOPLEX [3], a fully automated deep-learning tool combining transformer and U-Net architectures. The model was trained and fine-tuned on 20 manually segmented datasets from both MS patients and HC, acquired with the same MRI protocols. It outputs voxel-wise probability maps, with final segmentations generated using majority voting across the five top-performing networks to ensure robustness and reproducibility. All ChP masks were visually inspected by trained readers, and manual corrections were applied when needed. ChP volumes were normalized for head size to allow inter-subject comparisons and used to compute z-scores relative to age- and brain-size–adjusted normative values.
- **Genetic analysis.**
  - Genotyping was performed using Illumina® arrays, including: Human-660 Quad (N=34), HumanOmniExpress BeadChip (N=538), Global Screening Array (GSA, N=155) (640K–2.4M single nucleotide polymorphisms [SNPs]).
  - To account for differences across platforms, data were quality-controlled and imputed separately for each array type using the Michigan Imputation Server and the Haplotype Reference Consortium r1.1 panel. Standard QC filters were applied (sample call-rate ≥90%, heterozygosity within ±3 standard deviations [SDs], MAF ≥1%, HWE $p \geq 10^{-6}$), followed by identity-by-descent analysis to exclude related individuals (PI_HAT>0.25). Post-imputation, 8.29M SNPs passed quality filters and were merged into a single dataset.
- **Polygenic Risk Scores (PRS).**
  - **Human leukocyte antigen (HLA) genetic burden (HLAGB):** Calculated using 24 high-confidence HLA loci using well-established MS-associated loci identified by the International Multiple Sclerosis Genetics Consortium (IMSGC) [4], each weighted by the natural log of its odds ratio.
  - **Non-HLA PRSs:** Calculated using well-established MS-associated loci identified by the IMSGC [4]. SNPs in the extended HLA region were excluded to avoid overlap with the separate HLA genetic burden score (HLAGB). Variants were selected in three hierarchical PRSs: PRS1: 200 genome-wide significant SNPs; PRS2: PRS1 plus 117 strongly suggestive variants; PRS3: PRS2 plus 299 weakly suggestive variants. The final scores included 178 SNPs for PRS1, 281 SNPs for PRS2, and 533 SNPs for PRS3.
  - All scores were computed using PLINK v1.9, standardized, and adjusted for population substructure using the first five principal components derived from genome-wide principal component analyses (PCA).
- **Statistical analysis.**
  - In HC, normalized ChP volume trajectories were modeled with age, sex, and brain volumetric measures as predictors.
  - Individual z-scores were computed for MS patients to quantify deviations from normative values.
  - Associations with disease duration, EDSS, T2-hyperintense WM LV, brain volumes, and genetic scores were tested using robust regression, adjusted for multiple comparisons (Benjamini-Hochberg FDR).

RESULTS

- **Demographic features and volumetric associations in HC.**
  - In HC, higher NChP volume was significantly correlated with older age (r=0.323, p<0.001), lower NBV (r=−0.453, p<0.001), lower NCV (r=−0.330, p<0.001), NTV (r=−0.280, p<0.001), and NCaV (r=−0.141, p=0.002), as well as with higher NLVV (r=0.713, p<0.001). No sex-related differences in NChPV were observed (p=0.396).
  - Multivariable model predictors of NChPV: NBV (β=−1.11x10^{-3}, p=0.004), log-transformed NLVV (β=1.74, p<0.001) and its squared term (β=−1.53, p<0.001) (R^2=0.54). Demographics-only model explained less variance (R^2=0.16).
- **Volumetric trajectories across the adult lifespan.**
  - NBV and NTV: quadratic decreases, with significant losses beginning at ~30 and ~35 years, respectively (p-FDR<0.004) (**Figure 1A-1C**).
  - NCV and NCaV: linear declines across adulthood (p-FDR<0.001) (**Figure 1B-1D**).
  - NLVV: quadratic increase, with noticeable enlargement from ~35 years (p-FDR<0.007) (**Figure 1E**).
  - NChPV: stable until ~35 years, then non-linear increase accelerating in later decades (p-FDR<0.002) (**Figure 1F**).

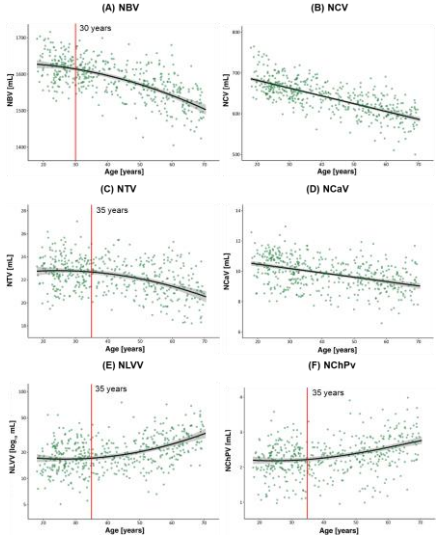

Figure 1. Lifespan trajectories of explored brain structure volumes in HC. (A) NBV; (B) NCV, (C) NTV, (D) NCaV, (E) NLVV, and (F) NChPV.

- **Demographic, clinical, and brain volumetric features in MS patients.**
  - **Table 1** shows the main demographic and clinical features in HC and MS patients.
  - Compared to HC, MS patients showed significantly higher normalized T2-hyperintense WM LV, NLVV, and NChPV (p-FDR<0.001). NBV, NCV, NTV, and NCaV were significantly lower in MS patients (p-FDR<0.001).
  - Compared to both RRMS and PPMS, SPMS patients showed significantly higher normalized T2-hyperintense WM LV and NLVV and lower NBV, NCV, NTV, and NCaV (p-FDR<0.001).
  - No significant differences in nChPV were observed among the MS phenotypes (p-FDR≥0.484).

	HC (n=461)	All MS (n=727)	p	RRMS (n=477)	SPMS (n=160)	PPMS (n=90)	RRMS vs HC p	SPMS vs HC p	PPMS vs HC p	SPMS vs RRMS p	PPMS vs RRMS p	SPMS vs PPMS p	p*
Demographic variables													
Sex, n (%)													
Male	210 (46)	281 (39)	0.022[a]	173 (36)	55 (34)	53 (59)	0.005[a]	0.018[a]	0.028[a]	0.736[a]	<0.001[a]	<0.001[a]	<0.001
Female	251 (54)	446 (61)		304 (64)	105 (66)	37 (41)							
Age [years]													
Mean (SD)	41.9 (15.1)	42.6 (11.3)	0.373[b]	39.1 (10.4)	48.9 (9.1)	49.9 (10.5)	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	0.470[b]	<0.001
(range)	(18.1;69.8)	(18.1;69.7)		(18.1;69.8)	(27.3;69.5)	(24.4;69.8)							
Clinical variables													
Disease duration [years]	-		-				-	-	-	<0.001[c]	0.014[c]	<0.001[c]	<0.001
Median (IQR)		10.5 (3.9;17.7)		8.0 (2.1;15.0)	18.9 (13.5;24.8)	9.5 (5.0;15.3)							
EDSS score													
Median (IQR)	-	2.5 (1.5;5.0)	-	1.5 (1.0;2.5)	6.0 (5.0;6.5)	6.0 (4.0;6.5)	-	-	-	<0.001[c]	<0.001[c]	0.100[c]	<0.001
DMT, n (%)#													
None		137 (19)	-	57 (12)	33 (21)	47 (52)	-	-	-	0.002[a]	<0.001[a]	<0.001[a]	<0.001
ME-DMT	-	361 (50)		272 (57)	68 (42)	21 (23)							
HE-DMT		229 (31)		148 (31)	59 (37)	22 (25)							

Table 1. Demographic and clinical features in HC and MS patients. aChi-square test; bWelch's t-test; cMann–Whitney U test. *Heterogeneity among MS clinical phenotypes assessed using the Chi-squared test (sex, DMT status), linear models (age), and Kruskal–Wallis test (disease duration, EDSS). #ME DMT=azathioprine, dimethyl fumarate, glatiramer acetate, methotrexate, interferon beta 1a, methotrexate, or teriflunomide; HE DMT=alemtuzumab, cyclophosphamide, cladribine, fingolimod, natalizumab, ocrelizumab, ofatumumab, rituximab, siponimod. Abbreviations: HE=high-efficacy; IQR=interquartile range; ME=moderate-efficacy.

- **Analysis of associations in MS patients.**
  - MS patients exhibited significantly higher NChPV z-scores than expected across the entire adult age range (18–70 years) (all p-FDR<0.001), with no evidence of age-dependent variation (p=0.957) (**Figure 2A**).
  - NChPV z-scores were already significantly elevated within the first year after clinical disease onset and continued to rise during the following four years (p-FDR=0.012–0.015), after which they plateaued (**Figure 2B**).
  - When stratified by disability status using an EDSS threshold of 3.0, higher NChPV z-scores were significantly associated with greater disability in patients with mild impairment (EDSS <3.0) (β=0.303, standard error [SE]=0.093, p=0.001), but not in those with EDSS ≥3.0 (β=−0.094, SE=0.052, p=0.070) (**Figure 2C**). The interaction was significant (p<0.001).
  - Higher nChPV z-scores were also significantly associated with higher normalized T2-hyperintense WM lesion volume (β=0.012, SE=0.004, p<0.001) (**Figure 2D**), whereas no significant associations were observed with normalized brain, cortical, thalamic, caudate, or lateral ventricle volume z-scores (all p>0.089).
  - No significant sex-related differences in nChPV z-scores were detected (p=0.275).

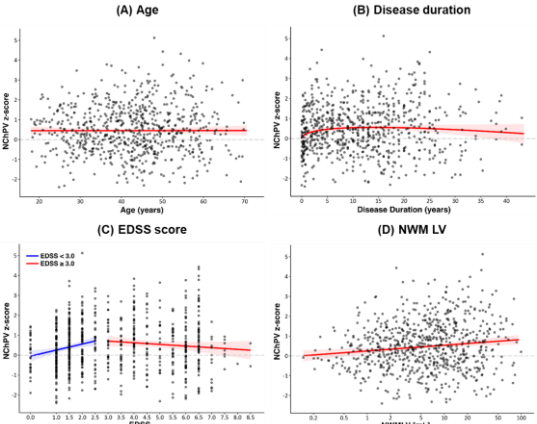

Figure 2. Normalized ChP volume z-scores associations with demographic, clinical, and MRI measures. Scatterplots with fitted regression lines and 95% confidence intervals illustrate the relationships between NChPV z-scores and: (A) age, (B) disease duration, (C) EDSS score, and (D) NWM LV. NChPV z-scores show no significant association with age but follow a nonlinear increase with disease duration, peaking around year 5. While no overall association is observed with EDSS, stratified analysis reveals a significant association in MS patients with EDSS <3.0 and no significant association in those with EDSS ≥3.0. A positive association with NWM LV supports a link between ChP enlargement and inflammatory burden. Abbreviations: NWM LV=normalized white matter lesion volume.

- **Associations between ChP volume and susceptibility genetic scores in MS.**
  - Higher HLAGB score was significantly associated with larger nChPV z-scores (standardized-β=0.097, SE=0.047, p=0.038). Among the 24 IMSGC-identified HLA loci evaluated, only *AA DQB1 221 32628784 exon4 Q* showed a nominally significant association with NChPV z-scores (β=0.149, SE=0.068, p=0.028), but this result did not survive FDR correction (p-FDR=0.452).
  - No significant associations were found for non-HLA PRSs, including PRS1 (standardized-β=0.024, SE=0.047, p=0.609), PRS2 (standardized-β=0.063, SE=0.047, p=0.177), and PRS3 (standardized-β=0.055, SE=0.047, p=0.241).
  - Neither HLAGB nor non-HLA PRSs were associated with normalized T2-hyperintense WM LV or other brain volumetric measures (p≥0.231).
  - A sensitivity analysis exploring HLAGB and each non-HLA PRS in combination confirmed that higher nChPV z-scores were significantly associated only with HLAGB (p≤0.036), but not with non-HLA PRSs (p≥0.159).
  - Extreme decile analysis supported these results since MS patients in the highest HLAGB decile had significantly larger nChPV z-scores than those in the lowest decile (estimated mean difference=0.478, SE=0.207, 95% confidence interval [CI]=0.071;0.885, p=0.022) (**Figure 3**), whereas no significant differences were observed between the top and bottom deciles of any non-HLA PRS (p≥0.089).

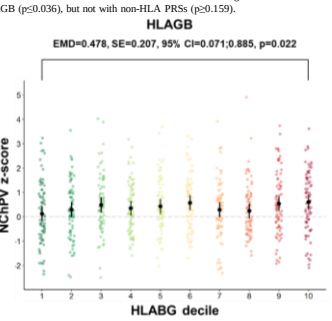

Figure 3. Distribution of NChP volume z-scores in MS patients stratified by deciles of HLAGB score. Dot-plot shows the distribution of NChPV z-scores across deciles of the HLAGB score, adjusted for age, sex, disease duration, EDSS, clinical phenotype, DMT status, as well as the first five eigenvectors from population sub-structure PCA. Black dots and error bars represent the estimated means and 95% CI, respectively.

CONCLUSIONS

- **MS patients exhibit significantly larger NChPV compared to HC, with enlargement occurring within the first year after symptom onset and plateauing after five years.**
- **Early ChP enlargement is associated with higher T2-hyperintense WM LV and HLA-related genetic risk, indicating a role for the ChP as a biomarker of inflammation and genetic susceptibility.**
- **These findings support a model in which the ChP contributes to MS pathogenesis via its role at the blood-CSF barrier.**
- **Our results highlight the potential of ChP volumetry as a novel, noninvasive biomarker of inherited susceptibility and active inflammation in MS.**

REFERENCES

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