Lateralization of TSPO Expression in the Human Brain: A PET Imaging Study Using [^{11}C]PBR28

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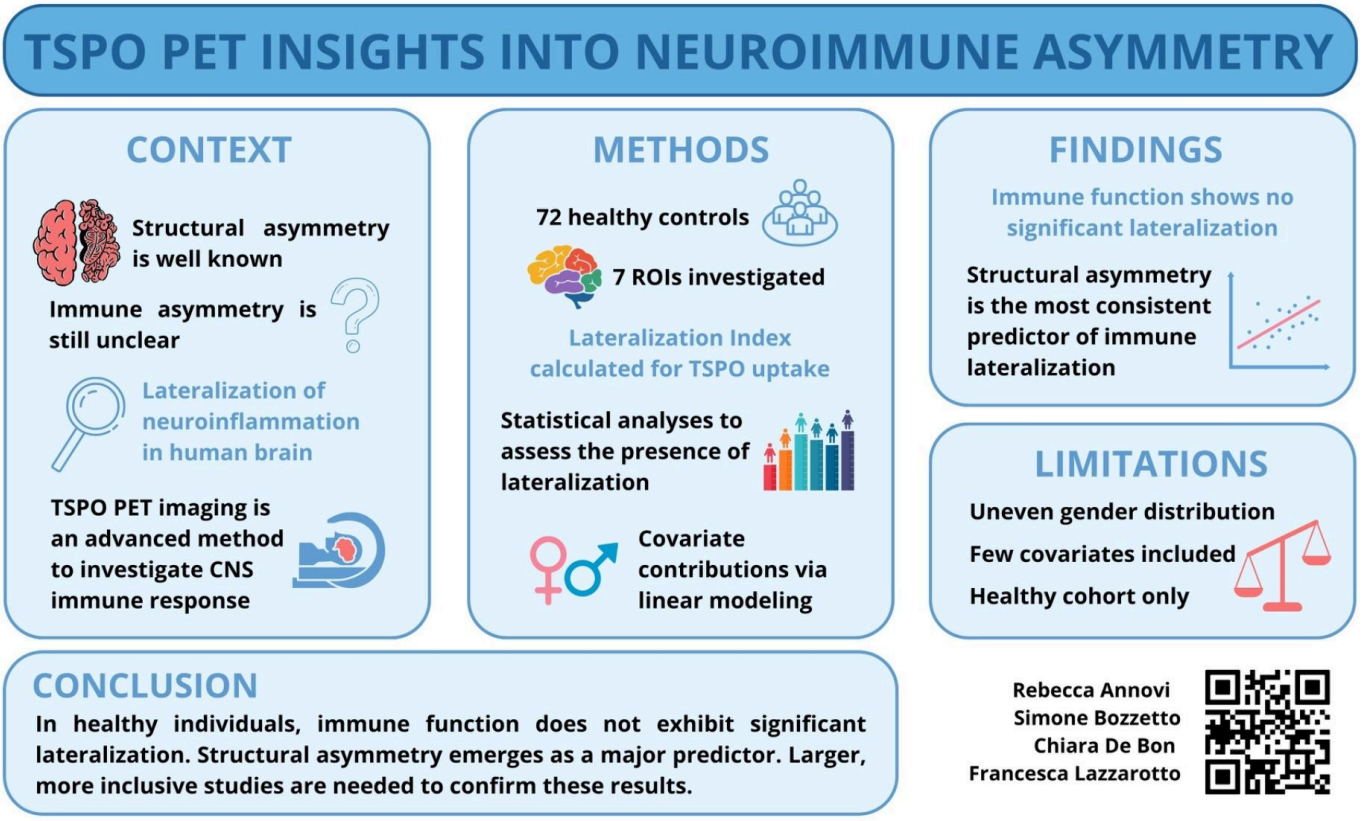
ABSTRACT

Neuroinflammation, measurable through PET imaging with TSPO translocator protein, is a key process involved in numerous neurological and psychiatric pathologies. The present study aims to investigate a possible lateralization of the expression of TSPO and to evaluate its association with variables such as genotype (HAB/MAB), age, and sex.
The Volume of distribution and Volume lateralization indices (respectively LI Vt and LI Vol) have been calculated in seven bilateral brain regions, in a 72 healthy subjects sample. The potential lateralization and the relationships between lateralization indices and demographic, genetic and structural variables have been explored through statistical analysis.
The study highlighted a general symmetry of the Volume of distribution lateralization index, further supported by the comparison of the raw volume of distribution values (Vt). Moreover, it revealed a discrepancy between LI Vt and LI Vol, indicating that structural asymmetry does not correspond to functional patterns.
In addition it has not been observed differences between sex and genotype LI Vt values, excluding a relative subgroup analysis. The regression models have identified LI Vol as the only consistent predictor of LI Vt, although with low explanatory power.
The limited sample size and the non balanced distribution of the demographic variables represent some limitations; future studies on clinical population could contribute to clarify the biological and clinical significance of TPSO lateralization.

VISUAL ABSTRACT



BACKGROUND

Neuroinflammation is a key process in various neurological and psychiatric disorders and has become a growing focus in neurobiological research. One of the most effective tools for studying immune activity in the brain is positron emission tomography (PET) imaging of the mitochondrial 18 kDa translocator protein (TSPO). However, it remains unclear whether TSPO expression in the human brain exhibits lateralization, which is an asymmetric distribution between the left and right hemispheres. Therefore, the present study aims to investigate the lateralization of neuroinflammation in the human brain using TSPO-PET imaging.

The 18 kDa translocator protein (TSPO) is a mitochondrial protein expressed by immune competent cells (macrophages, microglia, and astrocytes) and it increases in response to cellular injuries [1]. In the central nervous system (CNS), under physiological conditions, microglia usually exhibit a resting phenotype and can quickly switch to an activated phenotype in response to brain injury or inflammation. As a result, elevated TSPO expression has been consistently observed in neuroinflammatory conditions, correlating with microglial activation. Increasing evidence indicates that the progression of various neuropsychiatric and neurodegenerative disorders, including Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), major depressive disorder (MDD), and obsessive-compulsive disorder (OCD) are closely associated with neuroinflammation. Thus, TSPO has been considered a promising biomarker for neuroinflammation [2].

Lateralization refers to the anatomical, neurochemical, physiological and behavioral differences between the two hemispheres of the brain [3]. This specialization is thought to enhance cognitive efficiency by minimizing redundant processes, allowing each hemisphere to manage distinct tasks simultaneously [4]. In particular, as regards the asymmetric brain immune function, it is notable that the right hemisphere predominantly modulates the sympathetic nervous system, whereas the left hemisphere is more involved in parasympathetic regulation [3].

Evidence from studies on both humans and animals suggests that functional cerebral lateralization modulates immune responses in distinct and asymmetric ways [3]. This hypothesis is further supported by the high level of integration between the immune and nervous systems, which communicate through shared ligands and receptors [5].

For example, Bardos et al. [3] found that natural killer (NK) cell activity was significantly impaired following lesions to the left cortex, whereas right cortical lesions had no observable effect when compared to controls. Consistent with this idea, additional animal studies have shown that lesions to the left cortex suppress immune responses, including reductions in T-cell activity, mitogen responses, and antibody production. In contrast, right hemisphere lesions tend to enhance immune activity, increasing T-cell responses. These immunological asymmetries have been linked to both behavioral lateralization and hemispheric differences in neurotransmitter systems [5]. Complementary evidence from human clinical research supports these findings. In a pilot study of epilepsy surgery patients, left hemisphere resections were associated with decreased levels of lymphocytes and T cells in the early postoperative period, while right hemisphere resections produced an increase in these immune markers [5].

Despite extensive research has been conducted in this field, the exact mechanism of interaction between the brain and the immune system is not fully understood. Shen et al. proposed that cytokines, such as IL-1β and IL-6, may regulate immune responses through activation of the hypothalamic-pituitary-adrenal (HPA) axis, whose function is related to

brain lateralization. The results from this study, evaluated on mice, showed asymmetrical distribution of brain IL-6 in left-pawed animals and ambidextrous animals, but not in right-pawed animals, both in cortex and hippocampus. Furthermore, researchers found a positive correlation between IL-6 hemispheric distribution and the degree of behavioral lateralization both in cortex and hippocampus: IL-6 levels in left-pawed mice were higher in both left cortex and hippocampus, while ambidextrous animals had higher levels in the right part [6]. Since IL-6 is considered to play an immunosuppressive role in the central nervous system, it can be speculated that the immune-enhancing effects of the left hemisphere should be weakened in left-pawed mice, so they should be at a higher risk of immune disorders. Clinical studies have reported similar patterns in humans with a higher incidence of autoimmune diseases among left-handed individuals [3]. Notably, IL-6 also plays a key role in the brain's response to injury and is implicated in several neurodegenerative conditions (including Alzheimer's disease, Parkinson's disease, and other inflammatory CNS disorders), raising the possibility that asymmetries in IL-6 expression may influence the progression or presentation of these pathologies.

These connections may be particularly relevant in the context of neuroinflammatory processes, suggesting that lateralization should be considered when interpreting molecular imaging data, such as that obtained through TSPO-PET.

Regarding the evidence for TSPO brain PET left-right asymmetry, Gershen et al. [7] reported lateralization of TSPO expression in specific brain regions of patients with Temporal Lobe Epilepsy (TLE) and Hippocampal Sclerosis (HS). In particular, the study demonstrated significantly higher uptake of the radiotracer in the ipsilateral hemisphere to the seizure focus, indicating a consistent pattern of asymmetry. The hemispheric asymmetry index, calculated from TSPO PET data, has shown good test-retest reliability suggesting its use in clinical and research settings to monitor disease progression or response to therapeutic interventions targeting inflammation [8].

In conclusion, based on previous studies, it is reasonable to hypothesize that there is lateralization in TSPO brain expression. The purpose of this research is therefore to assess TSPO brain PET left-right asymmetry and to determine whether it is influenced by variables such as genotype (HAB and MAB), age, and sex of the patient.

MATERIALS AND METHODS

1. DESCRIPTION OF THE DATASET

In this study, PET scan data from 72 healthy subjects (healthy controls, HC) selected from the King's College London (KCL) were analysed, as described in Maccioni et al. [9]. Participants had no history of psychiatric or neurological disorders and were genetically classified according to the TSPO transporter polymorphism, resulting in 22 HABs (high-affinity binders) and 50 MABs (mixed-affinity binders). The mean age was 32.4 years (±13.1), with a gender distribution of 50 males and 22 females. All subjects underwent dynamic PET scanning with the TSPO protein-specific radioligand [^{11}C]PBR28. PET images were acquired using a Siemens Biograph™ TruePoint™ PET-CT scanner for a total duration of 90 minutes, divided into 26 frames of varying lengths (lasting from 15 seconds to 10 minutes). The acquisition also included a low-dose CT scan, used during preprocessing [9]. Following the preprocessing steps, the processed images were used to extract, for each brain region, the regional volume (Vol, in mm³) and the volume of distribution (Vt, in ml/cm³), the latter considered a proxy for TSPO density. The resulting dataset, which is the one used for the analysis in this report, is divided into 3 parts. The largest one includes Vt and Vol values for the following bilateral ROIs: thalamus, putamen, cerebellum, frontal lobe, temporal lobe, parietal lobe, and occipital lobe. The other two sections contain demographic and clinical data. More precisely, the clinical data contains brain volume and genotype, while the demographic data reports gender and age.

2. DATA QUALITY CONTROL

The quality of the given dataset was assessed by checking for missing values and none were found. Additionally, non-physiological values within the dataset were examined. In particular, as done by Masahiro Fujita et al. [10], a threshold of 10 ml/cm³ was set (for both HABs and MABs), corresponding to the mean value plus three standard deviations of Vt for the [^{11}C]PBR28 data [11]. It was also verified that the Vt, Vol and brain volume values were not negative, and that the sum of the regional brain volumes did not exceed the total brain volume for each subject. Two subjects showed abnormally high Vt and Vol values, which were considered non-physiological. This was confirmed by visually inspecting the statistically significant outliers through boxplot analysis: these two subjects were subsequently removed from the database before performing the statistical analysis.

3. DATA PREPROCESSING

To assess asymmetry in Vt and Vol values across cerebral ROIs, the Lateralization Index (LI) was calculated using the formula (1):

$$LI = \frac{Right\ Hemisphere\ Value - Left\ Hemisphere\ Value}{Right\ Hemisphere\ Value + Left\ Hemisphere\ Value} \quad (1)$$

This value is consistent with a previous study on asymmetry in Parkinson's disease (Kaasinen et al.) [12]. The index ranges from -1, indicating complete left dominance, to +1, indicating complete right dominance, with 0 representing perfect symmetry. In the LI formula, the denominator is essential as it acts as a normalization factor, making the index dimensionless and allowing for comparisons across subjects.

4. STATISTICAL ANALYSIS

Four separate analyses were conducted on the following set of measures:

- Lateralization Index of Vt (LI Vt);
- Raw values of Vt;
- Lateralization Index of Volumes (LI Vol);
- Raw values of Volumes.

Specific statistical methods were used for each of the four categories, based on the distribution of the data and the goals of the analysis. A two-sided p-value of 0.05 was used as the threshold of statistical significance and 95% confidence intervals were applied.
All analyses were performed using MATLAB, JASP, and R.

4.1. Normality Tests

Firstly, the normality of the data distribution was assessed in order to select the appropriate parametric or non-parametric tests and avoid interpretation errors. The tests used for this purpose were the Lilliefors test in MATLAB and the Shapiro-Wilk test in JASP.
Since the latter is considered more robust, its results were deemed more reliable (https://it.mathworks.com/matlabcentral/fileexchange/13964-shapiro-wilk-and-shapiro-francia-normality-tests). To support this choice, the swtest.m function, available on Mathworks File Exchange, was used in MATLAB, as the software does not implement the Shapiro-Wilk test.

4.2. Group comparison

For normally distributed data, an independent t-test was performed to compare the means between different groups. For the data that resulted non-normally-distributed, the non-parametric Mann-Whitney U test was used to determine whether the two groups shared the same distribution and, consequently, the same median.
The groups were classified based on the categorical variables of sex (male/female) and genotype (HAB/MAB). The comparisons were performed for each analysis.

4.3. Comparison between hemispheres

Paired t-test and Wilcoxon Signed Rank were used to analyze the raw values of Vt and Vol, with the purpose of comparing the values of the opposite hemispheres. Through this, the presence or absence of statistical differences between each side of the ROI was investigated.

4.4. Two-way Anova and Aligned Rank Transform (ART)

Two-way ANOVA was performed on the lateralization index of Vt in two ROIs that satisfied the assumptions of normality and homogeneity of variances, in order to evaluate possible interaction effects between categorical variables and determine whether both variables influenced the index. For the ROIs that did not meet the assumptions of ANOVA test, Aligned Rank Transform (ART) was performed using the same categorical variables.

5. LINEAR REGRESSION MODELS

To investigate the relationship between the Vt lateralization index and potential predictive factors, a linear regression model was implemented for each one of the seven ROIs. The models included, as independent variables, demographic and clinical information, and the Vol lateralization index, corresponding to the ROI under analysis. Coefficients were estimated using the least squares method, whereas to assess the goodness of fit of each model, the coefficient of determination (R^2) and error variance (σ^2) were calculated. Additionally,

standard error, coefficient of variation and residual analysis were used to evaluate the precision of the estimates.
To improve variable selection and reduce the risk of overfitting, the Elastic-Net regression was performed. The alpha parameter was set equal to 0.5, in order to balance the Lasso and Ridge components that penalize the linear model. Optimal coefficients and intercept were extracted after the selection of the lambda value that minimized the mean squared error.

RESULTS

This section presents the main findings regarding the asymmetry of TSPO PET signal (Vt) in healthy subjects, assessed using the Lateralization Index. Secondary analyses explore the association between LI and demographic, structural, and genetic variables.

1. LATERALIZATION OF TSPO PET SIGNAL

The LI of Vt was computed for each bilateral ROI. Visual inspection and summary statistics revealed that most LIs were close to zero, indicating general symmetry. However, variability across ROIs suggested potential asymmetries in specific regions.
To confirm these results, the paired t-test and Wilcoxon Signed-Rank test, made comparing the Vt of the two side ROI, showed significant hemispheric differences only in the temporal lobe ($p = 0.0324$), indicating an asymmetry. No significant differences were found in the thalamus, putamen, cerebellum, frontal, occipital and parietal lobes (all $p > 0.05$).

2. COMPARISON WITH VOLUMETRIC LATERALIZATION

The LI of volumes (LI Vol) was computed using the same formula seen for the lateralization of Vt. Normality tests indicated that most LI Vol values were normally distributed, except for the cerebellum. Wilcoxon test and paired t-test, evaluated on volumes both on the right and left ROIs, revealed significant asymmetries in all regions except for the frontal lobe. A graphical comparison of these asymmetries is shown in Figure 1 below.

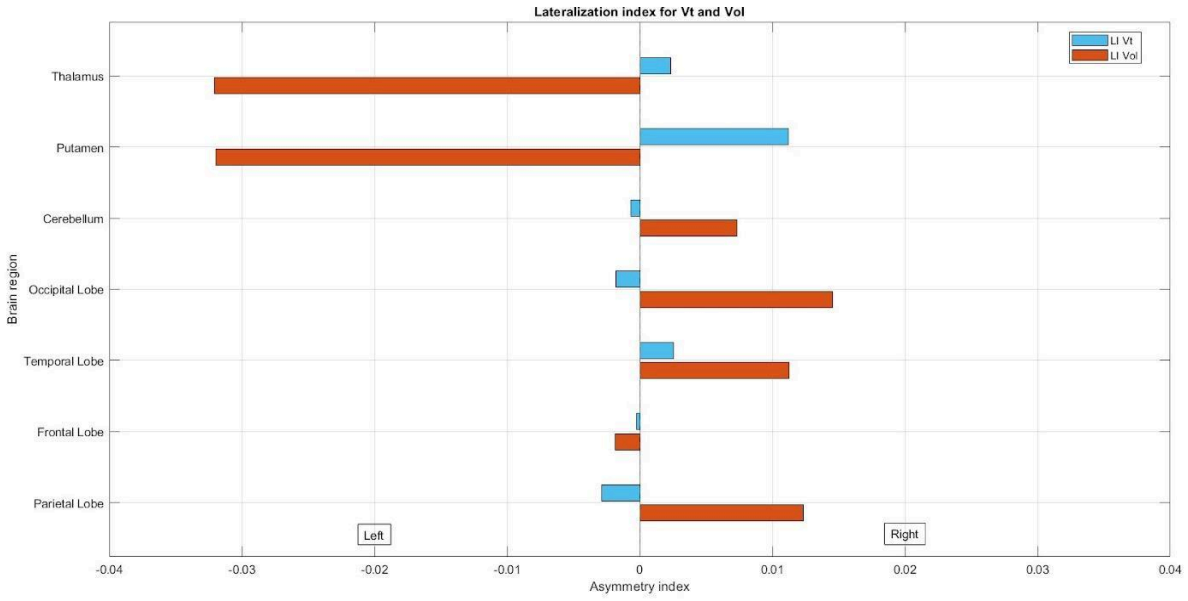


Figure 1. *The mean of the lateralization index for Vt, in blue, and Vol, in red, across the seven considered ROIs is shown, with the y-axis representing these values. The x-axis represents the asymmetry index, with negative values indicating leftward asymmetry and positive values indicating rightward asymmetry.*

3. EFFECT OF SEX AND GENOTYPE ON THE ASYMMETRIC INDEX

To investigate potential group effects, independent t-tests (for normal LI) and Mann-Whitney U tests (for non-normal LI) were used. Normality tests (Shapiro-Wilk) showed that only the LI Vt of the parietal and temporal lobes followed a normal distribution.

No significant differences were found in LI Vt between males and females or between HAB and MAB genotypes in the given ROIs. However, LI Vol revealed significant sex-related differences in the occipital lobe and cerebellum, as well as genotype-related differences in the occipital lobe and thalamus.

To evaluate whether there is a significant interaction between sex and genotype, a two-way ANOVA was performed only on the temporal and parietal lobes, as these were the only LI Vt distributions satisfying the assumptions of normality and homogeneity of variance. No significant interaction or main effects were found in either ROI (all $p > 0.05$).

The ANOVA based on the Aligned Rank Transform (ART) applied to non-normally distributed ROIs did not reveal significant correlations between the variables, except in the cerebellum, where a significant interaction was observed in MAB subjects based on sex, as seen in Figure 2.

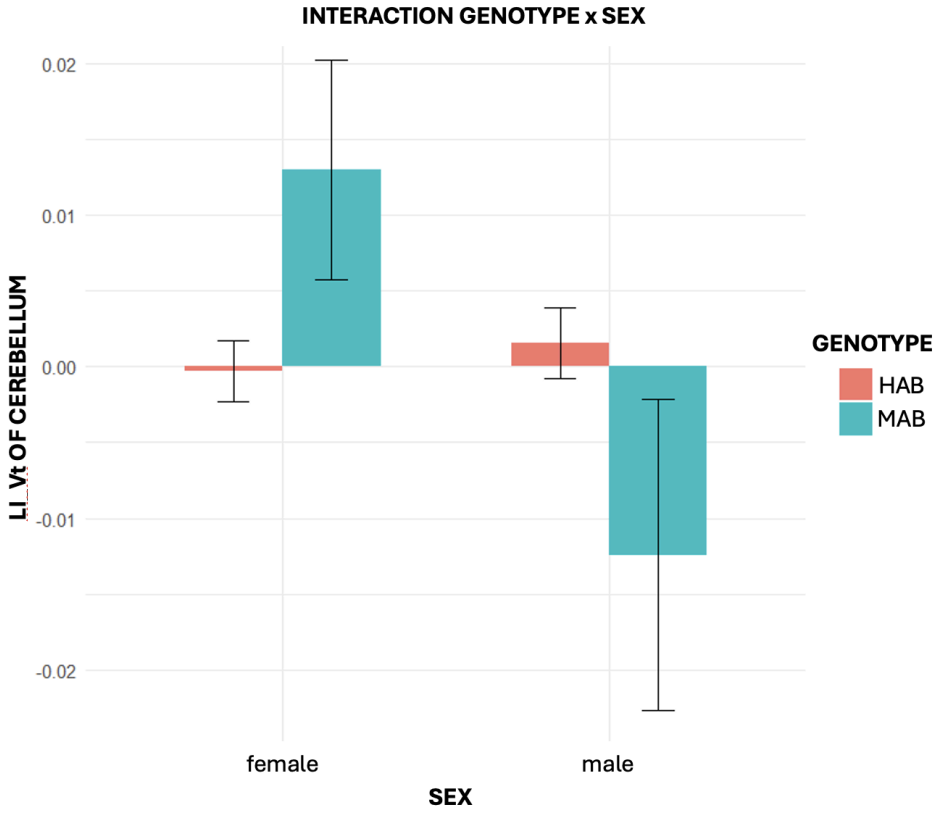


Figure 2. *Aligned Rank Transform ANOVA revealed a significant genotype × sex interaction in the cerebellum using Aligned Rank Transform ANOVA: MAB females showed higher LI Vt values than MAB males, with no sex difference in the HAB group. Error bars represent standard errors.*

4. REGRESSION ANALYSIS AND CONFOUNDING FACTORS

Linear regression models were run for each ROI, using age, sex, genotype, brain volume, and LI Vol as predictors. The coefficient of determination (R^2) ranged from 0.023 to 0.061. The most consistent predictor was the LI Vol of the corresponding ROI, especially in the thalamus and putamen.

Elastic Net regression, performed with α = 0.5, confirmed these findings by shrinking non-informative coefficients and retaining LI Vol in most models. Sex, age, brain volume and genotype were generally not retained.

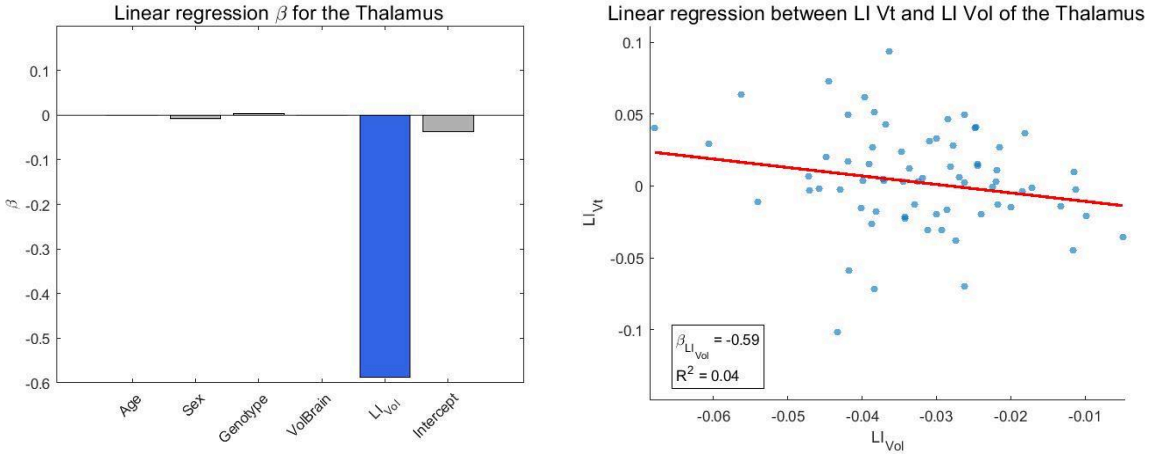


***Figure 3**. Linear regression analysis for the thalamus showing LI Vol as the strongest predictor of LI Vt, while age, sex, genotype, and brain volume had minimal influence. The scatterplot (right) illustrates a negative association between LI Vt and LI Vol (β = -0.59, R^2 = 0.04).*

5. VALIDATION VIA RAW VT VALUES

In addition to assessing hemispheric asymmetry through paired comparisons, raw Vt values were also analyzed to evaluate potential differences across sex and genotype groups. This extended analysis included tests of normality, homogeneity of variances, and group comparisons using appropriate statistical methods based on data distribution.

Shapiro-Wilk tests revealed non-normal distributions in most ROIs, prompting the use of Mann-Whitney U tests for between-group comparisons. These tests showed statistically significant differences between HAB and MAB genotypes (all $p < 0.05$), while for males and females half of the examined regions showed statistical differences.

DISCUSSION

These analyses revealed that most LI Vt values in the analysis being close to zero suggests a general symmetry, further supported by the comparison of Vt values between the two sides of each ROI, which showed significant differences only in the temporal lobe. This finding was also reported in the study conducted by Gershen et al. [7], which evaluated the presence of widespread TSPO overexpression using [^{11}C]PBR28 PET imaging in patients with temporal lobe epilepsy (TLE), comparing the results with healthy controls to exclude possible physiological left-right TSPO asymmetry. No significant left-right difference was observed in controls, consistent with what was previously demonstrated in the present study.

In contrast, LI Vol exhibited asymmetry in all regions except the frontal lobe, indicating volumetric differences between the left and right hemispheres. This highlights the lack of correlation between LI Vt and LI Vol, suggesting that structural asymmetry does not necessarily reflect asymmetry in TSPO expression.

The absence of significant differences in LI Vt values between sexes and genotypes indicates that subgroup analyses are not warranted. Consistently, a two-way ANOVA and ART ANOVA (sex × genotype) revealed no significant main effects or interactions in most of the ROIs ($p > 0.05$), suggesting that neither sex nor genotype influenced TSPO lateralization in these regions. The only significant interaction was found in the cerebellum, but this result should be interpreted with caution, as it was not supported by other statistical tests and may be attributed to the limited sample size.

In contrast, for raw Vt values, significant differences between genotypes and sexes were observed, in line with what was reported in the study by Jouni Tuisku et al [13], which showed, using [11C]PBR28 PET imaging on TSPO protein, significant differences in raw Vt values between males and females in all regions, with females presenting higher Vt. This divergence highlights how normalization via LI might obscure or neutralize between-group effects present in the raw signal. Therefore, analyzing Vt directly, allows to critically assess the limitations and strengths of LI as a metric, reinforcing that LI captures relative asymmetry but is not inherently sensitive to inter-group variability in absolute TSPO expression.

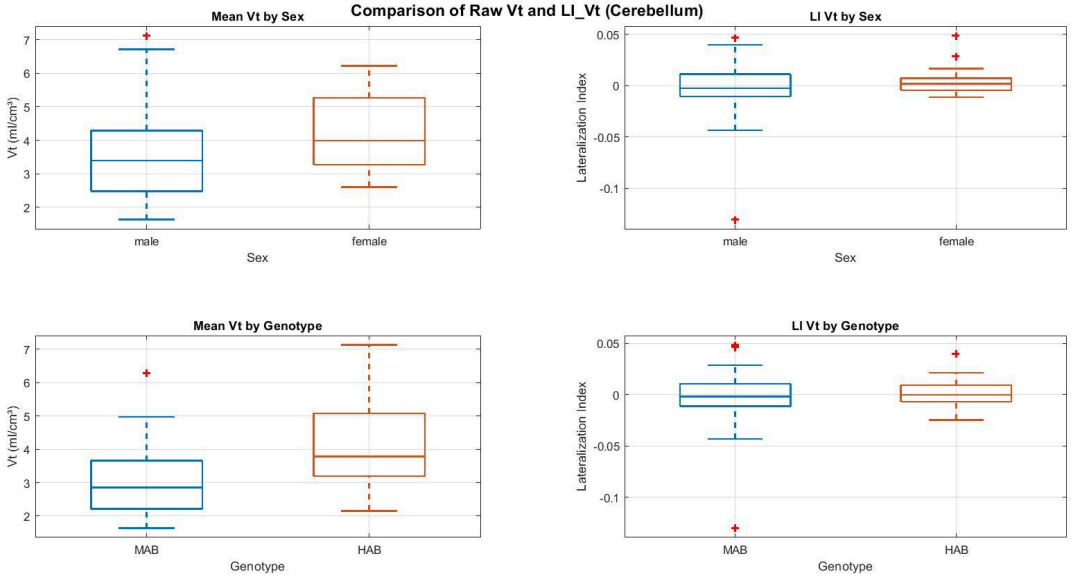


Figure 4. *Comparison of raw Vt and LI Vt values in the cerebellum, grouped by sex and genotype. Boxplots display the distribution of raw Vt (left) and LI Vt (right) for each group, illustrating the effects of group stratification on absolute and normalized PET measures.*

The comparison between the lateralization indices of Vt and Vol across all ROIs considered did not reveal any significant correlation. However, both linear regression and Elastic Net analyses identified the LI of Vol as the most consistent predictor of the LI of Vt, despite the low R^2 values, which suggest really poor explanatory power. This highlights the presence of a weak, but non-negligible, relationship between structural and functional lateralization.

This study presents some important limitations. The main one, which may affect the robustness of the results, is related to the sample size, which is relatively small and unbalanced in terms of sex, with only 22 females compared to 50 males. To overcome this issue, a cross-sectional study could be conducted using multiple datasets, in order to obtain a larger population sample. Secondly, only a limited number of covariates, such as age, sex and genotype, were included in the analysis. Further studies should consider additional variables that may help better explain the factors influencing TSPO lateralization. For example, the study conducted by Jouni Tuisku et al [13] also considered BMI, and found a significant relationship between BMI and Vt values.

Finally, our findings are limited to healthy subjects. Applying the same analysis also to clinical populations would help determine the clinical relevance of TSPO lateralization. For example, in the study conducted by Gershen et al. [7], it was found that patients with Temporal Lobe Epilepsy (TLE) showed lateralization of TSPO expression in specific brain regions.

In conclusion, the results demonstrate a general symmetry in TSPO expression as measured by LInVt, consistently with previous studies in healthy controls. This finding is in contrast with volumetric asymmetries observed in most regions, highlighting a dissociation between structural and functional lateralization. The absence of sex or genotype effects on LI Vt further supports the stability of TSPO lateralization in healthy individuals. However, significant group differences emerged when examining raw Vt values, revealing how normalization via lateralization indexes may obscure inter-group variability. Although no strong correlation was found between LI Vt and LI Vol, structural asymmetry showed a weak but consistent predictive value for functional asymmetry.

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