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Article

Impact of TSPO receptor polymorphism on [18F]GE-180 binding in healthy brain and pseudo-reference regions of neurooncological and neurodegenerative disorders

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Abstract: TSPO-PET tracers are sensitive to a single-nucleotide-polymorphism (rs6971-SNP) resulting in low (LAB), medium (MAB) and high (HAB) affinity binders, but the clinical relevance for [18F]GE-180 is still unclear. We evaluate the impact of rs6971-SNP on in vivo [18F]GE-180 binding in healthy brain and in pseudo-reference tissue in neurooncological and neurodegenerative diseases. Standardized uptake values (SUV) of [18F]GE-180-PET were assessed using a manually drawn region of interest in the fronto-parietal and cerebellar hemisphere. SUVs were compared between LAB, MAB and HAB in controls, glioma, 4-repeat tauopathies (4RT) and Alzheimer's disease (AD) subjects. Second, SUVs were compared between patients and controls within their rs6971-subgroup. After exclusion of patients with prior therapy, n=24 LABs (n=7 controls, n=5 glioma, n=6 4RT, n=6 AD) were analysed. Age- and sex-matched MABs (n=38) and HABs (n=50) were selected. LABs had lower fronto-parietal and cerebellar SUVs when compared to MABs and HABs, but no significant difference was observed between MABs and HABs. Within each rs6971 group, no SUV difference between patients and controls was detected in the pseudo-reference tissues. The rs6971-SNP affects [18F]GE-180 quantification, revealing lower binding in LABs when compared to MABs/HABs. Fronto-parietal and cerebellar ROIs were successfully validated as pseudo-reference regions.

Keywords: microglia; neurodegeneration; Alzheimer's disease; neurooncology; 4R-tauopathies; TSPO-PET

1. Introduction

The translocator protein 18 kDa (TSPO), previously known as the peripheral benzodiazepine receptor is a mitochondrial transporter involved in various intracellular processes. Its expression in the central nervous system (CNS) under physiological conditions is relatively low, but the expression is upregulated in activated microglia, macrophages and cancer cells [1]. TSPO received increasing importance as a positron-emission-tomography (PET) imaging target for several diseases, including CNS autoimmune diseases, neurodegeneration and glioma [2].

The first-generation TSPO-PET tracer [^{11}C]PK11195 has been used for over 25 years, though its application is limited due to poor pharmacokinetics and carbon-11 radiolabel [3-5]. Several second-generation TSPO ligands with an improved signal-to-noise ratio, including [^{11}C]PBR28 have been investigated and revealed substantial heterogeneity in binding potential due to inter-subject variability in the affinity for TSPO [6, 7]. Here, the binding properties of second-generation TSPO ligands have been found to depend on a genetic polymorphism in the TSPO gene. A single nucleotide polymorphism (rs6971), which replaces alanine by threonine (Ala147Thr), results in three patterns of binding affinity: high-affinity binders (HABs), mixed-affinity binders (MABs) and low affinity binders (LABs) depending on the homozygosity or heterozygosity of the allele [8]. Loss of binding to TSPO in approximately 10% LABs and underestimation of TSPO expression in LABs and MABs was reported [9]. Similar effects of the rs6971 polymorphism were also demonstrated in initial studies using [^{11}C]PBR28 [10, 11] and this phenomenon was subsequently noticed for several second-generation radiotracers [6, 11, 12]. Thus, the PET signal of patients with MAB and LAB status significantly underestimates TSPO expression and determination of the TSPO binding status is required. The development of next-generation TSPO tracers was a consequence of the sensitivity of the second-generation tracers to this polymorphism in the TSPO gene. Recently, the next-generation TSPO-PET tracer [^{18}F]GE-180 has received interest due to its fluorine labelling which makes the tracer available at centers without on-site cyclotron. Furthermore, recent studies reported a high lesion to background ratio, and a higher proportion of specific binding (45%) when compared to [^{11}C]PBR28 (33%) as shown by in vivo blocking [13-16].

Our group has studied [^{18}F]GE-180 in neurooncological, neurodegenerative and neuroimmune diseases. Glioma imaging with [^{18}F]GE-180 has shown to be valuable in non-invasive grading, with excellent sensitivity for the detection of high-grade gliomas [17-19]. [^{18}F]GE-180 imaging in patients with a clinical diagnosis of 4R-tauopathies (4RT) closely reflected the expected topology of microglial activation and indicated early detection in the disease course [20]. These findings have been underpinned by strong immunohistochemical correlations of CD68 staining and TSPO-PET signal in a Trem2 deficient amyloid mouse model and in tau transgenic P301S mice [21, 22]. However, clinical relevance of the rs6971 polymorphism on different binding affinity has not yet systematically been determined for [^{18}F]GE-180.

In this study we aimed to evaluate the impact of rs6971 on the in vivo [^{18}F]GE-180 signal in healthy brain and in potential pseudo-reference tissue for studies of neurooncological and neurodegenerative diseases.

2. Result

2.1. Sample composition and demographics

From a total of 380 participants, 113 participants were included in the analysis. Genotyping revealed 12% LABs (45/380) and after applying exclusion criteria 24 LABs consisting of 7 control subjects, 5 patients with glioma, 6 patients with a clinical diagnosis

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of a 4R-tauopathy and 6 patients with a clinical diagnosis of AD with a mean age of 67.9 years (95%-CI: 64.6-71.8) and a female to male ratio of 12:12 were included in the analysis. MAB and HAB patients were selected by the matching algorithm including minimal n, age, and sex **(Figure 1)**.

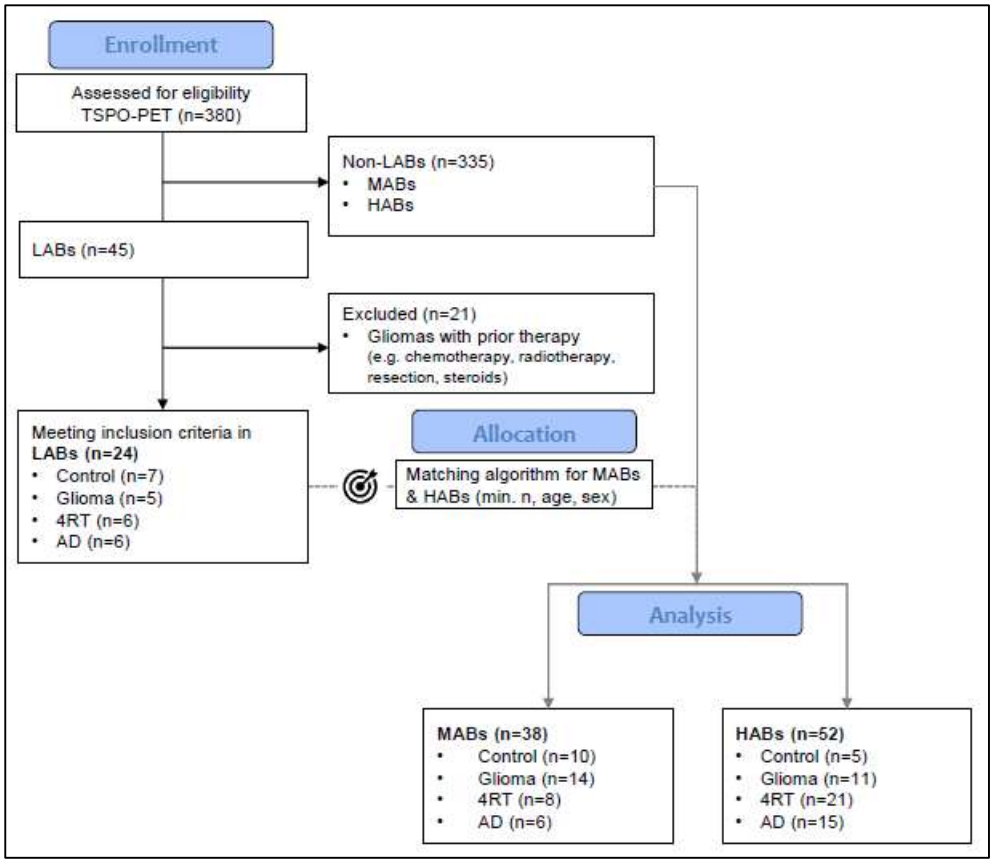

Figure 1. Flow chart of subject selection. LAB = low affinity binder; MAB = medium affinity binder; HAB = high affinity binder; AD = Alzheimer's disease; 4RT = 4-repeat tauopathies.

Thirty-eight MABs included 14 patients with glioma, 8 patients with a clinical diagnosis of a 4R-tauopathy, 6 patients with a clinical diagnosis of AD and 10 control patients with a mean age of 70.1 years (95%-CI: 67.2-72.9) and a female to male ratio of 23:15. Of 52 included HABs, 11 suffered from a glioma, 21 had a clinical diagnosis of a 4R-tauopathy, 15 had a clinical diagnosis AD and 5 were control subjects with a mean age of 70.4 (95%-CI: 67.9-72.8) and a female to male ratio of 23:29 **(Table 1)**. The diagnosis specific subgroups did not differ in binding status, age and sex. The glioma group consisted of 27 WHO Grade IV gliomas with an IDH-wildtype status and one WHO grade II glioma, IDH-mutant. The maximum standardized uptake values (SUV_{max}) of the tumour was 2.8 (95%-CI: 2.5-3.1) and the maximum tumour-to-background ratio (TBR_{max}) was 6.6 (95%-CI: 5.8-7.5). Analysed patients with 4R-tauopathies had a PSPRS score of 29.8 (95%-CI: 24.8-34.8), a MoCA score of 22.4 (95%-CI: 20.6-24-2), and a SEADL score of 60.6 (95%-CI: 54.2-67.1). Included patients of the AD-continuum had a MMSE score of 24.1 (95%-CI: 21.9-26.2), a global CDR score of 0.6 (95%-CI: 0.39-0.76) and a CDR sob score of 3.2 (95%-CI: 2.1-4.3). Controls did not show any signs of cognitive decline (MoCA 29.1 (95%-CI: 28.4-29.7)) or motor dysfunction.

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Table 1.

		LAB	MAB	HAB
Number of subjects		24	38	52
Diagnosis	Control (n)	7	10	5
	Glioma (n)	5	14	11
	4R-tauopathy (n)	6	8	21
	AD (n)	6	6	15
Age (y, mean, 95%CI)		68.2 (64.6-71.8)	70.1 (67.2-72.9)	70.4 (67.9-72.8)
Sex (♀ /♂)		12♀ / 12♂	23♀ / 15♂	23♀ / 29♂
Control	Age (y, mean, 95%CI)	68.4 (61.9-74.9)	69.1 (63.7-74.6)	72.2 (64.4-79.9)
	Sex (♀ /♂)	4♀ / 3♂	7♀ / 3♂	1♀ / 4♂
Glioma	Age (y, mean, 95%CI)	60.9 (51.7-70.0)	70.0 (64.6-75.5)	71.9 (65.7-78.1)
	Sex (♀ /♂)	2♀ / 3♂	8♀ / 6♂	4♀ / 7♂
	SUV_{max}	3.3 (2.4-4.1)	2.5 (2.0-3.0)	2.6 (2.1-3.2)
	TBR_{max}	8.7 (6.9-10.5)	6.2 (5.0-7.3)	6.2 (4.9-7.5)
4RT	Age (y, mean, 95%CI)	69.9 (62.4-77.3)	68.1 (61.7-74.6)	68.9 (64.9-72.8)
	Sex (♀ /♂)	4♀ / 2♂	5♀ / 3♂	10♀ / 11♂
	PSPRS	23.0 (3.0-42.9)	33.8 (23.8-35.1)	28.9 (22.7-35.1)
	MoCA	21.5 (14.1-28.9)	23.4 (19.7-27.1)	22.0 (19.8-24.3)
	SEADL	75.0 (49.3-100.7)	56.3 (43.4-69.1)	61.0 (53.0-68.9)
AD	Age (y, mean, 95%CI)	72.4 (65.4-79.3)	74.2 (67.3-81.1)	70.8 (66.4-75.1)
	Sex (♀ /♂)	2♀ / 4♂	3♀ / 3♂	8♀ / 7♂
	MMSE	23.4 (18.3-28.5)	23.3 (18.7-27.9)	24.6 (21.7-27.5)

	CDR	0.50 (0.06-0.94)	0.75 (0.35-1.15)	0.53 (0.28-0.79)
	CDR sob	3.8 (1.4-6.2)	4.6 (2.4-6.8)	2.5 (1.1-3.9)

Demographics at the group level. AD = Alzheimer's disease; CI = confidence interval; MMSE = mini-mental-state-examination; CDR = clinical dementia rating; sob = sum of boxes; SEADL = Schwab and England activities of daily living; PSPRS = progressive supranuclear palsy rating scale; MoCA = Montreal cognitive assessment; 4RT = 4-repeat tauopathies; SUVmax = maximum standardized uptake value; TBRmax = maximum tumour-to-background ratio; LAB = low affinity binder; MAB = medium affinity binder; HAB = high affinity binder.

2.2 [^{18}F]GE-180 binding in comparison of rs6971 polymorphism subgroups

Concordance between repeated quantification by manual region definition was excellent for the fronto-parietal (r=0.987) and the cerebellar (r=0.984) VOI. Overall LABs showed significantly lower SUVs in fronto-parietal and cerebellar VOIs when compared to MABs and to HABs **(Table 2)**. There was no significant difference observed between MABs and HABs **(Table 2)**. Specific findings in controls and disease groups are reported below.

Table 2.

	Frontal-parietal				Cerebellum			
Disease group	LAB (SUV ± SD)	MAB (SUV ± SD)	HAB (SUV ± SD)	p-value	LAB (SUV ± SD)	MAB (SUV ± SD)	HAB (SUV ± SD)	p-value
Control	0.345 ± 0.025	0.436 ± 0.021	0.471 ± 0.031	0.013* 0.006' 0.375#	0.367 ± 0.027	0.455 ± 0.023	0.514 ± 0.034	0.023* 0.004' 0.181#
Glioma	0.381 ± 0.021	0.424 ± 0.012	0.436 ± 0.014	0.086* 0.047' 0.511#	0.372 ± 0.028	0.463 ± 0.016	0.440 ± 0.018	0.009* 0.059' 0.338#
4RT	0.355 ± 0.028	0.455 ± 0.024	0.462 ± 0.015	0.010* 0.002' 0.826#	0.354 ± 0.032	0.475 ± 0.028	0.478 ± 0.017	0.007* 0.002' 0.930#
AD	0.360 ± 0.039	0.420 ± 0.039	0.435 ± 0.025	0.290* 0.118' 0.740#	0.397 ± 0.038	0.452 ± 0.038	0.460 ± 0.024	0.321* 0.175' 0.848#

Findings at one glance; TSPO PET quantification at the group level. Values represent regional group means of fronto-parietal and cerebellar VOIs and their standard deviation. *specific p-value for differences between the tracer uptake of LABs compared to MABs, 'LABs compared to HABs, #MABs compared to HABs. LAB = low affinity binder; MAB = medium affinity binder; HAB = high affinity binder; AD = Alzheimer's disease; 4RT = 4-repeat tauopathies; SUV = standardized uptake value; SD = standard deviation.

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2.1.1. Controls

The fronto-parietal SUV of LAB controls was significantly lower (0.345 ± 0.025) when compared to MAB controls (0.436 ± 0.021, p=0.013) and HAB controls (0.471 ± 0.031, p=0.006). A comparable effect was detected in the cerebellum, with a significantly lower SUVs of LAB controls (0.367 ± 0.027), compared to MAB controls (0.455 ± 0.023, p=0.023) and HAB controls (0.514 ± 0.034, p=0.004). There was no discernible difference of SUVs between MAB and HAB controls in both regions (fronto-parietal: p=0.375; cerebellum: p=0.181; **Figure 2**).

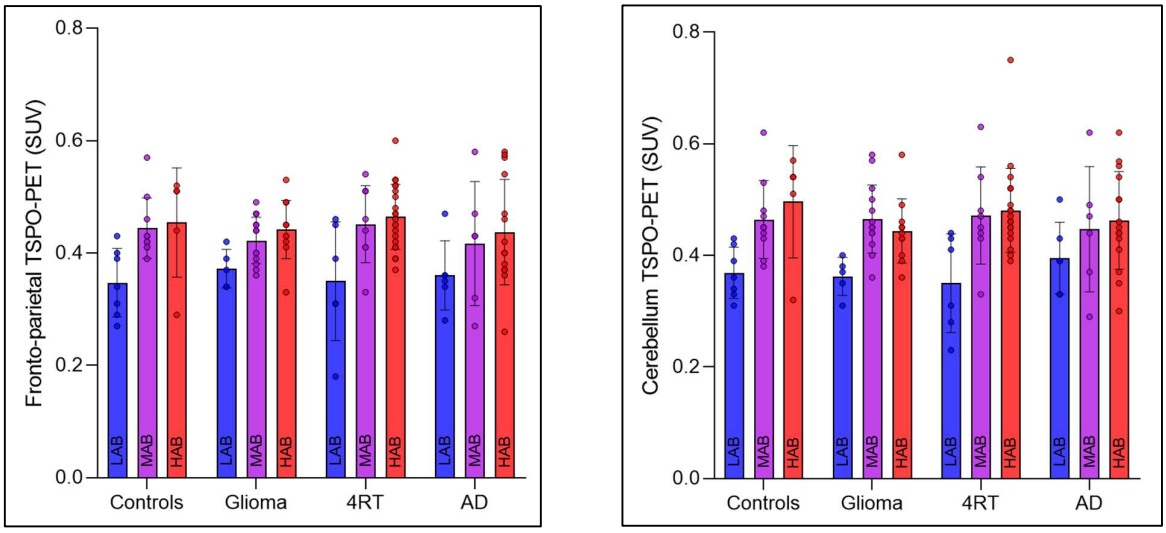

Figure 2. SUVmean distribution among the rs6971 polymorphism subgroups in controls and neurooncological and neurodegenerative diseases for the fronto-parietal VOI (A) and the cerebellar VOI (B). Error bars indicate standard deviation. LAB = low affinity binder; MAB = medium affinity binder; HAB = high affinity binder; AD = Alzheimer's disease; 4RT = 4-repeat tauopathies; SUV = standardized uptake value

2.1.2. Disease Group

2.1.2.1. Glioma

The fronto-parietal and cerebellar SUVs in the glioma cohort likewise differed significantly between the three TSPO binding polymorphism groups. The fronto-parietal VOI of LABs revealed significantly lower SUVs (0.381 ± 0.021) in comparison to MABs (0.424 ± 0.012, p=0.086) and HABs (0.436 ± 0.014, p=0.047).

The cerebellar VOI in the glioma group showed similar results with lower SUVs in LABs (0.372 ± 0.028) when compared to MABs (0.463 ± 0.016, p=0.009) and with a trend towards lower SUVs in HABs (0.440 ± 0.018, p=0.059). Again, no difference of SUVs between MAB and HAB glioma patients in both regions was detectable (fronto-parietal: p=0.551; cerebellum: p=0.338).

2.1.2.2. 4RT

In patients with 4R-tauopathies, we observed a significantly lower fronto-parietal SUV in LABs (0.355 ± 0.028) when compared to MABs (0.455 ± 0.024, p=0.010) and HABs (0.462 ± 0.015, p=0.002). In the cerebellum, the SUV was significantly lower in LABs (0.354 ± 0.032) when compared to MABs (0.475 ± 0.028, p=0.007) or HABs (0.478 ± 0.017, p=0.002). There was no difference between the SUV values in MAB and HAB 4R-tauopathy patients (fronto-parietal: p=0.826, cerebellar: p=0.930).

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2.1.2.3. AD

The results of patients with AD trended into the same direction as for other disease groups but they did not reach statistical significance. The fronto-parietal SUV of LABs was 0.360 ± 0.039, compared to MABs with a SUV of 0.420 ± 0.039 (p=0.290) and HABs with a SUV of 0.435 ± 0.025 (p=0.118). In the cerebellum, a non-significantly lower SUV was observed in LABs (0.397 ± 0.038), compared to MABs (0.452 ± 0.038, p=0.321) and HABs (0.460 ± 0.024, p=0.175). There was no discernible difference of SUVs between MAB and HAB AD patients for both regions (fronto-parietal: p=0.740; cerebellum: p=0.848).

2.3. [^{18}F]GE-180 binding in fronto-parietal and cerebellar pseudo-reference tissues within each rs6971 polymorphism subgroup

Visually, within each rs6971 polymorphism group, tracer binding of fronto-parietal and cerebellar VOIs was comparable between patients and healthy controls **(Figure 3)**.

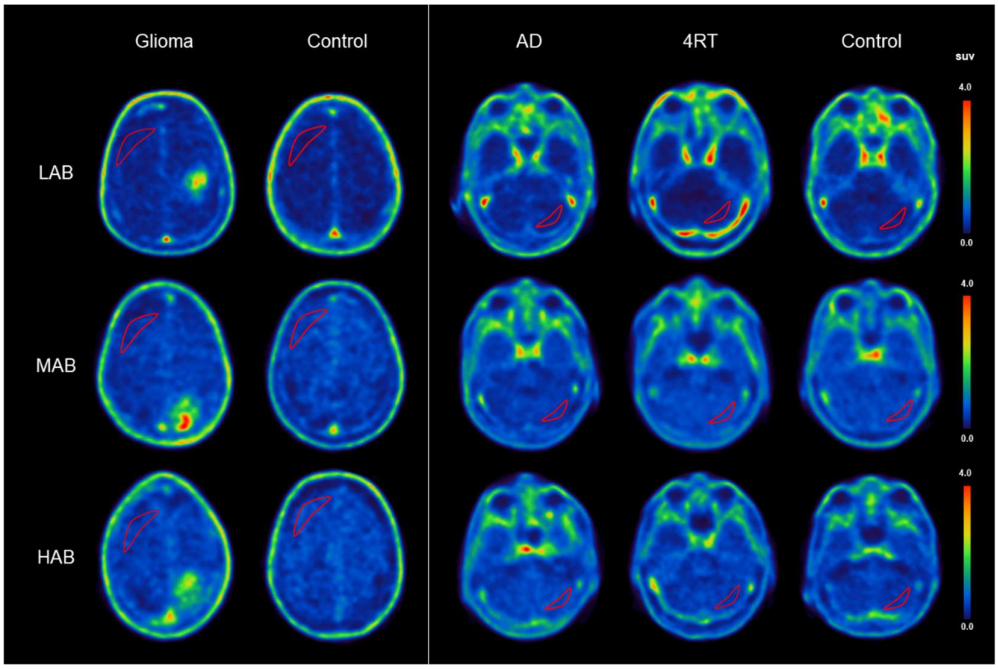

Figure 3. Differing [^{18}F]GE-180 uptake in the three polymorphism subgroups (LAB, MAB, HAB) but comparable intensities throughout the disease and control subjects within each rs6971 polymorphism subgroup. The crescent shaped red lines resemble the drawn ROIs for the pseudo-reference tissue assessment (as described in the methods). LAB = low affinity binder; MAB = medium affinity binder; HAB = high affinity binder; AD = Alzheimer's disease; 4RT = 4-repeat tauopathies; SUV = standardized uptake value

2.3.1. LABs

Within LABs, the fronto-parietal SUV as a potential pseudo-reference tissue for glioma TSPO-PET imaging, was similar between patients with glioma and controls (p=0.599). Similar results were obtained for the analysis of cerebellar SUVs which could be applied as a pseudo-reference tissue for TSPO-PET imaging of patients with glioma and neurodegenerative diseases. No significant difference was detected between all patient and control subjects (glioma vs. 4R-tauopathy p=0.817, glioma vs. AD p=0.174, glioma vs. control p=0.837, 4R-tauopathy vs. AD p=0.093, 4R-tauopathy vs. control p=0.626, AD vs. control p=0.184).

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2.3.2. MABs/ HABs

In the rs6971 polymorphism subgroups of MABs and HABs no significant difference of fronto-parietal SUVs was detected between patients with glioma and controls (p=0.405/ p=0.908). Cerebellar SUVs revealed similar results without significant differences between all patient and control subjects within MABs and HABs (glioma vs. 4R-tauopathy p=0.791/ p=0.220, glioma vs. AD p=0.567/ p=0.244, glioma vs. control p=0.977/ p=0.355, 4R-tauopathy vs. AD p=0.470/ p=0.987, 4R-tauopathy vs. control p=0.784/ p=0.936, AD vs. control p=0.608/ p=0.946).

2.4. Overall impact of the rs6971 polymorphism on [^{18}F]GE-180 binding

Finally, we aimed to determine the quantitative impact of the rs6971 polymorphism on [^{18}F]GE-180 SUV by a combined analysis of all study groups. Limited pathology in the cerebellum of all included subjects and limited pathology in the fronto-parietal region of patients with glioma and controls allowed us to estimate the rs6971 polymorphism impact in a robust fashion. The averaged difference between the SUVs of LABs compared to MABs was 19.0% ± 5.9% and the averaged difference between SUVs of LABs to HABs with 20.9% ± 5.3% **(Figure 4)**. The rs6971 impact was robust when considering the quantitative coefficient of variance of all four study groups.

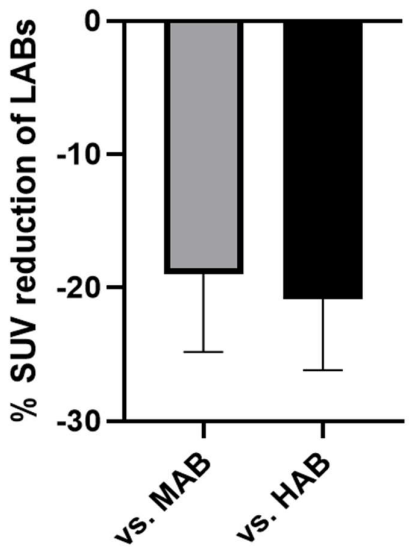

Figure 4. Percentage of [^{18}F]GE-180 SUV reduction of genetically determined LAB status. Mean values ± standard deviation derived from six comparisons (fronto-parietal: HC, glioma; cerebellum: HC, glioma, 4RT, AD) each. LAB = low affinity binder; MAB = medium affinity binder; HAB = high affinity binder; SUV = standardized uptake value.

3. Discussion

In this study, we investigated the tracer binding of [^{18}F]GE-180 in the three rs6971 polymorphism subgroups by an analysis in healthy controls, patients with glioma and patients with neurodegenerative diseases. The aim of this study was to determine if [^{18}F]GE-180 is sensitive to the rs6971 polymorphism as known from other TSPO radioligands and to evaluate potential pseudo-reference tissues for simplified clinical application of this tracer.

The main strength of our study consists of a meaningful sample of 24 LABs after exclusion of relevant confounding factors such as radiotherapy, chemotherapy, or steroids. Overall LABs showed a significantly lower [^{18}F]GE-180 SUV compared to MABs and

HABs and the impact of a LAB status was estimated to a SUV reduction of 19%/ 21%, respectively. In a study using post-mortem tissue for in vitro tracer binding, the TSPO-PET radioligands, PBR28 and PBR06 showed a more pronounced difference in binding affinity with a 50-fold and 17-fold higher affinity of HABs compared to LABs, respectively. PBR111 and DPA713 indicated a 4-fold higher affinity of HABs compared to LABs and PK11195 showed a negligible difference in binding affinity (0.8-fold) of HABs compared to LABs [6, 23]. The purpose of our study was to evaluate the impact of the rs6971 polymorphism on [^{18}F]GE-180 application in vivo. In line with many of the aforementioned studies, we found a sensitivity of this tracer for the rs6971 polymorphism. In conclusion, LABs need to be considered carefully when performing TSPO-PET imaging with [^{18}F]GE-180 in neuro-oncology and neurology, since the rs6971 polymorphism significantly impacts quantification. From the current perspective, LABs should be excluded and the inclusion of MABs and HABs should be feasible with consideration of the rs6971 polymorphism as a covariate, given the minor differences in binding among MABs and HABs.

The validity of [^{18}F]GE-180 as an in vivo read-out of microglial activation has been discussed extensively and with a focus on the question if [^{18}F]GE-180 uptake is mainly driven by blood brain barrier disruption [24-27]. In this regard, [^{18}F]GE-180 revealed a high signal-to-noise ratio in preclinical studies, but low brain penetration in human healthy controls [15, 28]. Missing rs6971 polymorphism differences in disease [17, 29] have been one major argument to question the specificity of [^{18}F]GE-180 binding to the microglial TSPO receptor. Our study demonstrates for the first time a significant rs6971 polymorphism impact on the [^{18}F]GE-180 uptake in vivo, thus refuting this earlier criticism[26]. In line with our data, Sridharan et al. performed a blocking study to quantify the specific binding of [^{18}F]GE-180 to TSPO and measured a 45% specific signal, concluding that despite low brain penetration, [^{18}F]GE-180 exhibits a specific signal in the brain [16]. Furthermore, our translational study in P301S mice and patients with 4R tauopathies indicated microglia specific uptake of [^{18}F]GE-180 in a depletion experiment and no dependency of the tracer uptake by markers of blood brain barrier integrity. Microleakage was claimed as a potential source of [^{18}F]GE-180 signal elevation in regions without MRI contrast enhancement [18, 19, 26, 30]. Our current data did not show an elevated [^{18}F]GE-180 signal in pseudo-reference regions of patients with glioma or neurodegenerative diseases when compared to healthy controls, regardless of the rs6971 polymorphism status. Thus, a general disease related microleakage as the main driver of the [^{18}F]GE-180 signal seems unlikely. In summary, our data enhance the evidence for a specific [^{18}F]GE-180 signal in vivo.

The second analysis of the current study questioned the use of potential pseudo-reference regions for [^{18}F]GE-180 PET imaging. Molecular imaging in neuro-oncology is most commonly performed with amino acid tracers, such as [^{18}F]FET. Our group has evaluated the fronto-parietal hemisphere as a favorable pseudo-reference tissue for the quantification of the [^{18}F]FET tumour uptake in clinical routine [28]. However, a concomitant neuroinflammatory response in brain regions without tumour infiltration could potentially affect TSPO-PET binding in a contralateral pseudo-reference tissue. Here, [^{18}F]GE-180 SUVs in patients with glioma were not different in comparison to healthy controls within each polymorphism subgroup. Thus, [^{18}F]GE-180 SUVs did not indicate an impact by the presence of glioma in the contralateral hemisphere. Studies with other TSPO radioligands reported that TSPO expression in gliomas is predominantly related to neoplastic cells and lacking expression of TSPO in surrounding reactive astrocytes and we note that further research using step-wise stereotactic biopsies in spatial correlation to the PET is warranted to elucidate the issue of general neuroinflammation in the presence of glioma further [31-33]. Taken together, our results support the use of the fronto-parietal region as pseudo-reference region for neurooncological imaging with [^{18}F]GE-180 in analogy to [^{18}F]FET, at least at primary diagnosis prior to any therapeutic intervention.

The cerebellum is frequently used as a pseudo-reference tissue in PET imaging of neurodegenerative diseases due to low disease burden and only limited β-amyloid and tau pathology in postmortem samples of AD and 4R tauopathies, with exception of the dentate nucleus [34-36]. In addition, our current study did not indicate significant differences of [^{18}F]GE-180 SUVs in the cerebellum of patients with neurodegenerative diseases when compared to controls within each polymorphism subgroup. This qualified the cerebellum as a suitable pseudo-reference region for TSPO-PET imaging of neurodegenerative diseases using [^{18}F]GE-180. In line, others also suggested the cerebellum as a suitable pseudo-reference region for [^{11}C]PBR28 imaging of AD [37]. Taken together, our [^{18}F]GE-180 analysis in dedicated rs6971 polymorphism subgroups revealed that potential pseudo-reference tissues for neurooncological and neurodegenerative diseases do not show altered binding in disease when compared to controls, thus making them suitable for relative quantification. Noteworthy, we also did not find significant increases of [^{18}F]GE-180 binding in the fronto-parietal VOI of patients with AD or 4R-tauopathies when compared to healthy controls, although microglial activation in these cortical areas is known in AD and 4RT. However, our previous work in 4R-tauopathies indicated that cortical TSPO-PET signal elevation is phenotype dependent and regionally heterogeneous in individual patients with maximum VOI differences of ~15% at the group level [38]. Thus, it was not surprising that the less robust measure of SUV (in terms of variance) did not reach a significant group difference between 4RT and controls in a manually drawn VOI. It remains to be tested if pseudo-reference region normalization facilitates concomitant use of LAB data together with MAB/HAB data in disease. This will depend on the presence or absence of disease specific binding in target regions of labs and the investigation will require similar patient cohorts per rs6791 polymorphism subgroup.

Limitations of the study need to be considered. Our results were based on static [^{18}F]GE-180 scans acquired from 60 to 80 minutes p.i. without arterial blood sampling. Thus, we were not able to consider the impact of kinetic modelling, tracer plasma availability and tracer metabolism in different rs6971 polymorphism subgroups. However, given the low incidence of LABs, we note that large numbers of participants with dynamic scanning and/or arterial sampling would be required to address such questions. In this regard, stable time-activity curves after 30 minutes p.i. have been shown in the healthy tissue of former studies [19, 30]. Another limitation is the small sample size of several of our subgroups, which, however, is also related to the generally low incidence of LABs. Thus, the statistical power was not sufficient to include several covariates that have been shown to be associated with TSPO-PET binding, such as obesity [39].

The rs6971 polymorphism has an impact on [^{18}F]GE-180 quantification in vivo, leading to 19%/ 21% reductions of SUV in LABs when compared to MABs/-HABs. Fronto-parietal and cerebellar pseudo-reference regions can be used for patients with glioma and neurodegenerative diseases.

4. Methods

4.1. Study design, study population and clinical assessments

The study and the data analyses (ethics-applications: 17-569, 17-755, 17-656 & 19-022) were approved by the local ethics committee (LMU-Munich, Germany). Genotyping was performed for all subjects that received a TSPO-PET at the department of nuclear medicine of the University Hospital of the Ludwig-Maximilians-University (LMU) Munich. Due to the disproportional distribution of the rs6971 polymorphism, controls and patients with glioma, Alzheimer's disease (AD) and 4RTs were first screened for LABs. Age- and sex-matched MABs and HABs for controls and all disease groups were included to secure a homogenous study population using a step-wise demographic guided selection algorithm. All glioma patients with prior therapies (chemotherapy, radiotherapy) were excluded a priori. All subjects with immunomodulatory therapies (i.e.

steroids) were excluded a priori. Patients with newly diagnosed and later confirmed glioma were included in the glioma cohort. Diagnosis of 4R-tauopathies was made according to the revised Armstrong Criteria of probable CBS or the Movement Disorders Society criteria of possible/probable PSP or possible PSP with predominant CBS [40, 41]. The AD-continuum patients were required to meet criteria for typical AD with mild cognitive impairment or dementia according to the diagnostic criteria or the National Institute on Aging and the Alzheimer's Association [41]. Exclusion criteria were severe neurological or psychiatric disorders other than AD-continuum or 4R-tauopathies. Healthy controls had no evidence of cognitive impairment following a neuropsychological battery which included the ADAS-Cog, a CDR score of 0, no family history of AD or neurological disease association with dementia and no objective motor symptoms. From all available MAB and HAB cases, the algorithm excluded mismatched cases (in terms of LAB-matching) until age and sex were indifferent between rs6971 groups ($p>0.1$). The algorithm was stopped for group sizes ≤5.

4.2. TSPO Genotyping

All individuals underwent genotyping for the genetic polymorphism of the TSPO gene and were classified as LAB, MAB or HAB. As previously described [19] whole-blood samples were sent to the Department of Psychiatry of the University Hospital Regensburg for polymorphism genotyping. Genomic DNA was extracted from 4 ml of whole blood using a QIAamp DNA Blood Maxi kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA quality was assessed by optical absorbance and gel electrophoresis. Exon 4 of the TSPO gene and exon/intron junctions were amplified by PCR and sequenced using the Sanger method with the following primers: ex4-F-AGTTGGGCAGTGGGACAG and ex4-R-GCAGATCCTGCAGAGACGA. Sequencing data were analysed using SnapGene software (GSL Biotech; http://snapgene.com).

4.3. TSPO-PET acquisition and analysis

TSPO-PET scans were acquired with a Biograph 64 PET/CT scanner (Siemens, Erlangen, Germany) at the Department of Nuclear Medicine, LMU Munich. A low-dose computed tomography scan preceded the PET acquisition and served for attenuation correction. Automated production of [^{18}F]GE-180 was performed as published previously [42]. After injection of 189±12 MBq [^{18}F]GE-180, all patients and controls received a static 60-80 min p.i. scan [20]. The respective summation images were used for image analysis [18, 19]. Images were reconstructed using OSEM3D algorithm (8 iterations, 4 subsets, 4 mm Gauss). For each scan, standard corrections for attenuation, scatter, decay, and random counts were applied.

According to the prior evaluated and published method for background activity assessment for [^{18}F]FET in glioma [43], a merged VOI consisting of five manually drawn, crescent-shaped ROIs in the fronto-parietal lobe including white and grey matter were assessed in the contralateral side of the tumour.

For patients with neurodegenerative diseases a manually-drawn cerebellar VOI (HC, 4R-tauopathies, AD) was used, to avoid β-amyloid or tau positive supratentorial regions. Five manually drawn ROIs, containing ~2 ml each, were drawn in a crescent shape in the posterior lobe of the cerebellum, excluding the vermis, the anterior lobe, the peduncle and the flocculus to ensure sufficient distance to any vessels and to exclude regions involved in disease. All five ROIs were merged to a single VOI for both regions.

For comparison reasons, both VOIs were drawn in all groups. In order to evaluate intra-reader variability, each patient was evaluated five times by a single operator. Concordance between repeated quantification by manual region definition was

calculated. Group comparisons of [^{18}F]GE-180 SUVs between patients with glioma, 4R-tauopathy or AD and controls as well as within each TSPO polymorphism group were performed by an analysis of variance (ANOVA) with the significance level $p < 0.05$ using age and sex as covariates.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of LMU-Munich, Germany (ethics-applications: 17-569, 17-755, 17-656 & 19-022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: M.B. received speaker honoraria from GE healthcare and LMI and is an advisor of LMI. G.U.H. has ongoing research collaborations with Prothena; serves as a consultant for AbbVie, AlzProtect, Asceneuron, Biogen, Biohaven, Lundbeck, Novartis, Roche, Sanofi, UCB; received honoraria for scientific presentations from AbbVie, Bial, Biogen, Bristol Myers Squibb, Roche, Teva, UCB, and Zambon; and holds a patent on PERK Activation for the Treatment of Neurodegenerative Diseases (PCT/EP2015/068734). C.H. is chief scientific advisor of ISAR biosciences and collaborates with DENALI therapeutics. R.P. is on the advisory board for Biogen, has consulted for Eli Lilly and Roche, is a grant recipient from Janssen Pharmaceutica and Boehringer Ingelheim, and has received speaker honoraria from Janssen-Cilag, Pfizer and Biogen. J.L. reports speaker fees from Bayer Vital and from Roche, consulting fees from Axon Neuroscience, author fees from Thieme medical publishers and W. Kohlhammer GmbH medical publishers, non-financial support from Abbvie and compensation for duty as part-time CMO from MODAG GmbH, all outside the submitted work. All other authors do not report a conflict of interest.

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