# Influence of *TSPO* Genotype on $^{11}$C-PBR28 Standardized Uptake Values

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$^{11}$C-PBR28 binds to the high-affinity state of the translocator protein 18 kDa (TSPO). A single-nucleotide polymorphism (rs6971) within the human *TSPO* gene determines the affinity state of the TSPO. The rs6971 genotype determines whether individuals express the high-, low-, or mixed-affinity phenotype of TSPO. The rs6971 genotype corresponds to *in vivo* $^{11}$C-PBR28 binding, as measured quantitatively by total volume of distribution. However, it is not known whether standardized uptake value (SUV) can detect differences in brain $^{11}$C-PBR28 uptake by *TSPO* genotype. **Methods:** Thirty-two older adults (71.8 ± 7.94 y old) underwent $^{11}$C-PBR28 PET scanning; rs6971 genotype was imputed after genomewide genotyping. SUV was extracted for several brain regions. The sample included 19 C/C carriers (high-affinity phenotype), 12 T/C carriers (mixed-affinity), and 1 T/T carrier (low-affinity) for rs6971. **Results:** SUV was 30% lower in T/C subjects than in C/C subjects. **Conclusion:** The results indicate that brain $^{11}$C-PBR28 SUV is sensitive to *TSPO* genotype.

**Key Words:** $^{11}$C-PBR28; positron emission tomography; TSPO; genotype

**J Nucl Med 2013; 54:1320–1322**
DOI: 10.2967/jnumed.112.118885

The translocator protein 18 kDa (TSPO) is part of a cholesterol transport complex on the outer mitochondrial membrane. Because overexpression of TSPO is associated with microglial activation, several TSPO PET ligands have been developed to study neuroinflammation in neurologic and psychiatric disorders, including $^{11}$C-PK11195, $^{11}$C-DA1106, $^{11}$C-DPA713, $^{11}$C-PBR28, $^{18}$F-PBR06, and $^{18}$F-FEPPA (*1–6*). $^{11}$C-PBR28 stands out in the field of available TSPO ligands because of an exceptionally high affinity at the TSPO and an excellent signal-to-noise ratio (*7*). In fact, studies with $^{11}$C-PBR28 have led to a better understanding of the *in vivo* state of the TSPO. In previous studies, approximately 10%–20% of humans did not show appreciable specific binding of $^{11}$C-PBR28 (*3,7,8*). This observation led to the discovery that humans exhibit 1 of 3 TSPO binding profile phenotypes: high, low, and mixed affinity (*7,9*). Subsequently, it was found that a single-nucleotide polymorphism in the *TSPO* gene (rs6971) is associated with *in vitro* affinity of the TSPO in platelets (*10*). Regardless of TSPO radioligand, identification of subjects' TSPO affinity phenotypes via *in vitro* binding assays or genotyping is critical for proper interpretation of TSPO PET data. Ascertaining TSPO affinity phenotypes is also necessary to eliminate low-affinity subjects and to balance groups for high-affinity and mixed-affinity subjects. Additionally, for any given TSPO tracer, it is equally important to characterize how well the selected method of data analysis can discriminate among the affinity phenotypes or genotypes. It has been shown that *in vitro* TSPO affinity in leukocytes corroborates with the quantitative metric of total volume of distribution ($V_T$) of $^{11}$C-PBR28 (*7*). This work was recently extended to the demonstration of a direct correspondence between rs6971 genotype and *in vivo* brain $^{11}$C-PBR28 $V_T$ (*11*). Estimation of $V_T$ requires arterial sampling, which is invasive, can be painful, and may be difficult in populations such as the elderly. A potential alternative index of $^{11}$C-PBR28 uptake is the semiquantitative standardized uptake value (SUV), which does not require arterial sampling. However, it is not known whether *TSPO* genotype (rs6971) is similarly predictive of brain $^{11}$C-PBR28 SUV. Here, we present evidence that $^{11}$C-PBR28 SUV is sensitive to differences in $^{11}$C-PBR28 uptake between subjects with the high-affinity and mixed-affinity TSPO phenotype, as identified by rs6971 genotype.

## MATERIALS AND METHODS

All procedures were approved by the Indiana University Institutional Review Board. All subjects signed a written informed consent form. The participants (mean age ± SD, 71.8 ± 7.94 y) were a subset of a larger multimodal neuroimaging study and included healthy older adults ($n = 9$) and individuals with diagnoses of cognitive complaints in the absence of significant cognitive deficits ($n = 9$), mild cognitive impairment ($n = 7$), or Alzheimer disease ($n = 7$). Previous publications by Saykin et al. (*12*) and others (*13,14*) provide additional information about the diagnostic criteria. To determine study eligibility, the subjects underwent several neuropsychologic and clinical assessments, as previously described (*12*). Qualified subjects subsequently underwent imaging procedures and provided a blood sample for genotyping.

An anatomic 3-dimensional magnetization-prepared rapid-acquisition gradient-echo MR imaging sequence was acquired on a 3-T Tim Trio (Siemens) (*15*). $^{11}$C-PBR28 was synthesized as described previously (*16*). Dynamic PET scans (HR+; Siemens) were initiated with injection of approximately 555 MBq of $^{11}$C-PBR28 (Table 1). Data were acquired for 90 min (10 × 30 s, 9 × 60 s, 2 × 180 s, 8 × 300 s, 3 × 600 s). PET data were motion-corrected and normalized to Montreal Neurological

Received Dec. 18, 2012; revision accepted Mar. 18, 2013.
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Published online Jun. 19, 2013.

**TABLE 1**
Subject and Scan Characteristics by rs6871 Genotype

| Characteristic | C/C | T/C | T/T |
|---|---|---|---|
| *n* | 19 | 12 | 1 |
| Mean age (y) | 70.9 ± 7.32 | 73.9 ± 8.84 | 64 |
| Sex | 6 men | 3 men | Male |
| Race | 1 African-American; 18 Caucasian | 3 African-American; 9 Caucasian | All Caucasian |
| Diagnosis | 5 CON, 7 CC, 2 MCI, 5 AD | 3 CON, 2 CC, 5 MCI, 2 AD | CON |
| Mean activity injected (MBq) | 524 ± 74.7 | 497 ± 93.1 | 506 |
| Mean mass dose (nmol/kg) | 0.23 ± 0.14 | 0.23 ± 0.15 | 0.11 |

AD = Alzheimer disease; CC = cognitive complaint; CON = control; MCI = mild cognitive impairment.

Institute space, similarly to previously published methods (*17*). Static images were created from data between 35 and 90 min. SUV images were produced according to the standard definition of SUV: the amount of radioactivity in the static image was divided by the injected dose of $^{11}$C-PBR28 per total body weight. Subject-specific regions of interest for cerebellar cortex, thalamus, caudate, putamen, brain stem, and parietal cortex were generated from each subject's anatomic MR images using Freesurfer, version 4.0.1. Average $^{11}$C-PBR28 SUVs were extracted for each region of interest.

Genomewide genotyping was performed using the HumanOmniExpress BeadChip kit (Illumina); standard quality control procedures were conducted as described previously (*18*). The rs6971 genotype was imputed using IMPUTE, version 2.2, with the 1000 Genomes phase 1 data as a reference panel. A posterior probability of 0.90 was the threshold for acceptance of the imputed genotype.

Independent *t* tests were used to check for group differences in age, $^{11}$C-PBR28 dose parameters, and $^{11}$C-PBR28 SUV from each region of interest.

## RESULTS

Nineteen participants were homozygous for the major allele of rs6971 (C/C; high-affinity TSPO site). Twelve subjects were heterozygous (T/C; mixed-affinity phenotype). There was 1 homozygous minor allele carrier (T/T; low-affinity phenotype). Subject demographics are listed in Table 1. C/C and T/C groups were not significantly different with respect to age and were reasonably well-balanced for sex, race, and diagnoses. There were no group differences in injected radioactivity or mass dose (Table 1).

$^{11}$C-PBR SUVs for the C/C and T/C groups are shown in Figure 1. Data from the T/T carrier are presented for illustrative purposes. There were significant group differences in SUV in all regions. The significance levels for each region of interest were as follows: cerebellum, $P < 0.00005$; thalamus, $P < 0.02$; caudate, $P < 0.003$; putamen, $P < 0.0008$; brain stem, $P < 0.02$; and parietal cortex, $P < 0.00003$. On average, T/C carriers had a 30% lower signal than the C/C group. The small sample size did not permit statistical assessment of any genotype X diagnosis interactions. Figure 2 shows individual SUVs for the thalamus by genotype, with each subject's data point identified by diagnosis. The distribution of diagnoses was similar for all regions.

## DISCUSSION

This study provides evidence that $^{11}$C-PBR28 SUV is strongly associated with the rs6971 single-nucleotide polymorphism in the *TSPO* gene. In our sample, rs6971 genotype conferred a 30% difference in SUV between the high-affinity and mixed-affinity *TSPO* carriers. This finding is consistent with data reported by

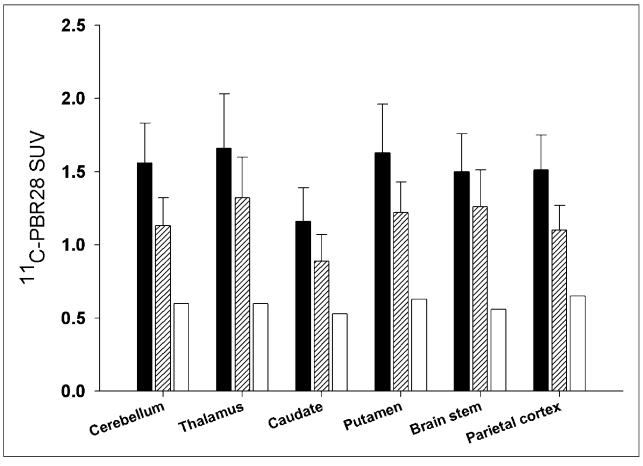


**FIGURE 1.** Average $^{11}$C-PBR28 SUV for C/C (*n* = 19; black bars), T/C (*n* = 12; hatched bars), and T/T (*n* = 1, white bars) *TSPO* genotypes. Data are mean ± SD (except for T/T subject). In all brain regions, T/C group had statistically significantly lower SUV than C/C group (independent *t* test). *P* values are provided in "Results" section.

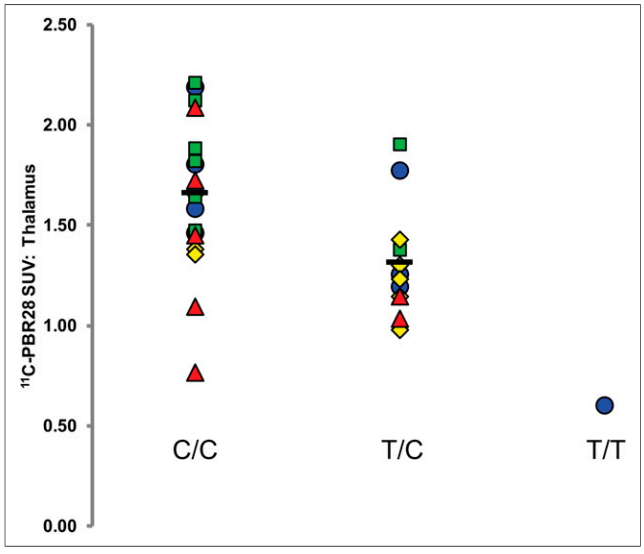


**FIGURE 2.** Individual thalamic $^{11}$C-PBR28 SUVs for all subjects, by genotype (C/C, left; T/C, middle; C/C, right) and diagnosis. ○ = controls; □ = cognitive complaint; ♢ = mild cognitive impairment; △ = Alzheimer disease; bars = mean thalamic SUV for genotype.

Kreisl et al. (*11*), in which the $V_T$ of brain $^{11}$C-PBR28 was approximately 25%–35% lower in T/C than in C/C subjects (depending on brain region). The ability to detect TSPO affinity phenotype differences in this modest sample suggests that $^{11}$C-PBR28 SUV measurements may be a reasonable alternative to absolute quantitative kinetic modeling methods that require arterial plasma sampling. However, this study did not directly compare SUV with the gold standard, $V_T$. Although the current dataset suggests that the use of SUV is a promising approach, future modeling studies with arterial sampling and estimation of $V_T$ will be necessary to characterize and validate the use of SUV as a dependent variable in $^{11}$C-PBR28 studies.

## CONCLUSION

We present preliminary evidence that SUV measurements of $^{11}$C-PBR28 binding are capable of distinguishing between TSPO affinity phenotypes in humans. The sensitivity of $^{11}$C-PBR28 SUV for detection of diagnostic or treatment-related effects on TSPO binding may be greatly enhanced by controlling for rs6971 variation. Even with group matching for TSPO affinity phenotype, more work is needed to estimate the minimum detectable effect size when $^{11}$C-PBR28 is the primary outcome variable. Regardless of analysis method, power will likely improve with the development of a correction factor for the *in vivo* imaging data that accounts for relative proportions of high-affinity and low-affinity sites (*19*).

## DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734. This work was supported, in part, by grants from the National Institute on Aging (R01 AG19771 and P30 AG10133), the Indiana Clinical and Translational Sciences Institute (U54 RR025761), the NCRR (construction grant C06-RR020128), and the National Library of Medicine (K99 LM011384). No other potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

We thank Dr. Robert Innis of the NIMH for assistance with implementation of $^{11}$C-PBR28 synthesis and Drs. Mark Green, Clive Brown-Proctor, and Qi-Huang Zheng for radionuclide production, radiopharmaceutical synthesis, and quality assurance. Drs. Martin Farlow, Brenna McDonald, and Darren O'Neill participated in diagnostic assessments, and Dr. Gary Hutchins provided helpful feedback on data analysis. We also acknowledge Barbara Glick-Wilson and Brandon Steele for technical assistance with tracer production, Larry Corbin for engineering support, Tamiko MaGee for subject recruitment, John West for computer support, and Kevin Perry, Michele Beal, and Courtney Robbins for PET and MR image acquisition.

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*J Nucl Med.* 2013;54:1320-1322.
Published online: June 19, 2013.
Doi: 10.2967/jnumed.112.118885



*The Journal of Nuclear Medicine* is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)



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