Human Subjects
Healthy subjects originated from two clinical protocols conducted at the Service Hospitalier Frédéric Joliot, Orsay, France (NCT02305264 and NCT02319382). The PET session was identical for both clinical protocols. These studies were conducted according to French legislation and European directives with the approval of the Medical Bioethics Committee of the Ile de France Region. Eligibility was determined from medical history and physical examination: exclusion criteria were active medical conditions, history of CNS or psychiatric disease. No anti-inflammatory or benzodiazepine medications known to interfere with TSPO were taken for at least 3 weeks before the study. None of the participants had contraindications excluding them from MRI, and for women of child bearing age, the risk of pregnancy was omitted by a negative serum pregnancy test obtained the day of the PET exam.

Genomic DNA from blood samples was used to genotype the rs6971 polymorphism within the TSPO gene in all individuals. DNA was extracted from blood samples, PCR amplification of the TSPO gene was carried out and PCR products were purified with magnetic beads Ampure® XP (Agencourt®) and sequenced with an Applied Biosystems 3730xl DNA analyser. The genetic analysis revealed seven HABs and three MABs.

MRI Protocol
T1-weighted MR images were acquired for all subjects using either a Philips (Best, The Netherlands) Achieva 1.5T MR scanner using turbo fast echo sequence (TE/TR =3/6.3 ms; alpha = 10, resolution=0.92*0.92*0.93 mm) or a Siemens Trio 3T (Erlangen, Germany) scanner using a 3D MPRAGE (TE/TI/TR=2.98/900/2300, alpha=9, resolution=1x1x1.1 mm).

18F-DPA-714 Synthesis and PET Protocol
18F-DPA-714 (N,N-diethyl-2-(2-(4-(2-18F-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide) was prepared according to standard conditions (1) using a TRACERLab™ FX-FN synthesizer (GEMS, France). Radiolabelling of DPA-714 with fluorine-18 uses a tosloxy-forfluorine nucleophilic aliphatic substitution (one-step process) and its preparation includes the following five stages: 1) dilution of the no-carrier-added, dried (activated) K[18F]-Kryptofix®222 complex (prepared from [18F]fluoride, potassium carbonate and Kryptofix®222) with 700 μl of dimethylsulfoxide containing 3.5 to 4.5 mg of the tosloxy precursor for labeling (N,N-diethyl-2-(2-(4-(2-toluenesulfonyloxyethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide); 2)-
incubation of the reaction mixture at 160°C for 5 min; 3)- dilution of the reaction mixture with the HPLC mobile phase and pre-purification on a SepPak® Alumina N™ cartridge; 4)- HPLC purification on a semi-preparative Waters X-Terra™ C-18 column (eluent 0.1M aq. ammonium acetate (pH 10) / acetonitrile : 60 / 40 (v:v)); and 5)- SepPak® Plus C-18 cartridge-based removal of the HPLC solvents. A range of 9.6 to 11.2 GBq of 18F-DPA-714 (15% ethanolic solution in saline, 10 mL-volume batches) is routinely obtained within 50-55 min. The non-decay-corrected isolated yield typically ranges between 30-35% with specific radioactivity between 74 to 222 GBq/μmol and a radiochemical purity higher than 99%.

Subjects underwent 18F-DPA-714 PET in a 3D high resolution research tomograph (HRRT, Siemens, Knoxville, TN, USA). The HRRT is a dedicated brain imaging system with a 31.2 cm transaxial and 25.5 cm axial FOV (207 image slices of 1.2-mm thickness). After a transmission scan using a 137Cs point source, an 18F-DPA-714 bolus was injected intravenously (201.4±26.3 MBq). The dynamic PET acquisition in list mode lasted 90 min.

A radial artery was catheterized by an anaesthesiologist after the application of local anaesthesia using a lidocaine/prilocaine (2.5%) transcutaneous patch (EMLA®). During PET scan acquisition, sequential arterial blood was manually sampled (n=21) to generate an arterial plasma input function (AIF). Ten sequential venous blood samples were also withdrawn. The fractional parent radioactivity was determined in arterial and venous samples (5, 10, 20, 40, 60, 70 and 90 min) as follows: blood samples were immediately centrifuged at 3500 rpm at 4°C for 5 min to obtain cell free plasma. After centrifugation, the plasma radioactivity was measured in a cross-calibrated gamma-counter (Cobra Quantum D5003; Perkin-Elmer, USA). 18F-DPA-714 was isolated from the radiometabolites in plasma using a previously described solid phase extraction method (2). Briefly, after deproteinization, the plasma was loaded on a 60 mg HLB Oasis™ SPE cartridge (Waters, France) and the unchanged 18F-DPA-714 was eluted. The radioactivity measured in this eluate was expressed as a fraction of the total plasma radioactivity taking into account the recovery coefficient of the whole procedure. The unchanged parent fraction in arterial and venous blood samples was plotted versus time and fitted (mono-exponential decay) to generate the metabolite-corrected plasma curves. The AIF was calculated as the linear interpolation of the concentrations of unchanged 18F-DPA-714 before the peak and as a tri-exponential fit of concentrations after the peak. We defined the corrected vein samples as the venous function (VF).

18F-DPA-714 PET Quantification
After correction for attenuation and random scattered coincidences, PET images were reconstructed with the iterative ordered-subset expectation maximization (Ordinary Poisson [OP]-OSEM) 3D method. Dynamic data were binned into 27 time frames (6×1 min, 7×2 min, 14×5 min). A 3D Gaussian kernel with 2 mm full-width at half-maximum was used as a point-spread function correction for the spill-in and spill-over.

In the simplification method, we estimated the equilibrium state between the parent plasma “P” and the free ligand “F” compartments (and between free “F” and bound “B” ligand compartments) using the ratio of the concentrations in these two compartments (F/P and B/F). Indeed, the transfer rates between the P and F compartments (and between F and B compartments) are linear and therefore, the equilibrium is reached when the ratio F/P (and B/F) becomes constant, equal to K1/k2 (and k3/k4).

**PBIF Method**

PBIF is a method that has been proposed to reduce the number of blood samples to only one or two arterial (3) or venous (4) samples. When creating the standard input function (SIF), delays between individual curves were adjusted so that the peak for all subjects matched. \( T_a \) was chosen as the arterial sample time with the highest correlation between the AUC and the arterial value at different times (3). The PBIF\(_{\text{artery}}\) was estimated for each subject according to PBIF\(_{\text{artery}}\) = SIF × AIF\(_T\). To replace the arterial sample with the venous samples for the normalization of the SIF, we defined a linear function \( f(x) \) using \( AIF'_{T_a}=f(VF) \), where \( VF \) is the average activity in the vein samples between 60 and 90 minutes. This function was estimated using both arterial and venous samples of the SIF subjects, according to a leave-one-out approach. The estimated venous PBIF for each subject was then computed as PBIF\(_{\text{vein}}\) = SIF × \( f(VF) \) = SIF×AIF\(_T\).
SUPPLEMENTAL REFERENCES


Supplemental Figure 1

Time-activity-curves expressed in SUV in different ROIs across the three genetic groups: HABs, MABs (mean value ± SD) and one LAB.