Limited predictive power of DAT mRNA mappings for DAT protein expressions

Kyoungjune Pak (✉ ilikechopin@me.com)

  Pusan National University School of Medicine https://orcid.org/0000-0001-5051-1894

Seongho Seo

  PNU: Pusan National University

Myung Jun Lee

  PNU: Pusan National University

Hyung-Jun Im

  SNU: Seoul National University

Keunyoung Kim

  PNU: Pusan National University

In Joo Kim

  PNU: Pusan National University

Original research

Abstract

Background

Dopamine transporter (DAT) is a transmembrane protein that translocates dopamine from the extracellular space into the presynaptic neurons. We aimed to investigate the predictive power of DAT mRNA for DAT protein expression measured from positron emission tomography (PET).

Methods

Thirty-five healthy subjects were imaged with ^{18}F-FP-CIT PET. Binding potential (BP_{ND}) from ventral striatum, caudate nucleus, putamen, middle frontal, orbitofrontal, cingulate, parietal, temporal cortices were measured. DAT gene expression investigated in this study was obtained from the freely available Allen Human Brain Atlas (www.brain-map.org) derived from 6 healthy donors.

Results

PET-derived BP_{ND}s from 35 subjects, and DAT mRNA expressions of 6 probes from 6 subjects were included in this study. The auto-correlation of PET-derived BP_{ND}s for DAT was intermediate (mean ρ^2 = 0.6588) with ρ^2 ranging from 0.0811 to 1. However, the auto-correlation of mRNA expression was weak across the probes with mean ρ^2 from 0.0889 to 0.2263. Cross-correlations between PET-derived BP_{ND}s, and mRNA expression were weak with mean ρ^2 ranging from 0.0011 to 0.2220 across the probes.

Conclusion

we observed weak associations between DAT mRNA expressions, and DAT protein expressions in human brains. Therefore, there might be a limited predictive power of DAT mRNA mappings for DAT protein expressions in humans.

Background

Dopamine is a neurotransmitter that plays a major role in reward-motivated behavior, and motor control (1). Brain dopamine neurotransmission is regulated by dopamine transporter (DAT), a transmembrane protein that actively translocates dopamine from the extracellular space into the presynaptic neurons in dopaminergic system (2). Dysfunction of DAT has been known to be linked to neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder (3), biopolar disorder (4), and alcoholism (5). In addition, DAT is a major target for various pharmacologically active drugs (2).

Allen Human Brain Atlas is a freely available multi-modal atlas of gene expression and anatomy of all brain regions (6). Six postmortem brains were analyzed for microarray-based expression, in situ hybridization gene expression, and also magnetic resonance imaging-based brain mapping (6). Previously, the predictive power of brain mRNA mappings of Allen Human Brain Atlas was reported after comparison with positron emission tomography (PET)-derived protein expressions including serotonin receptor (7–9), serotonin transporter (8, 9), opioid receptor (7), monoamine oxidase A (MAO-A) (9, 10). Therefore, we measured striatal DAT availability using dynamic positron

emission tomography (PET) scans with ^{18}F-FP-CIT, a high-affinity radioligand for DAT from a study by Pak et al (11) to measure DAT protein expression.

We hypothesized that DAT mRNA expression can predict DAT protein expression measured from PET scans. Therefore, we investigated the predictive power of DAT mRNA expression with 1) auto-correlation, and 2) cross-correlation with PET-derived DAT protein expression.

Methods

PET data

Thirty-five healthy, male subjects without brain injury, neuropsychological disorders were included in this study. The majority of the participants in this study were included in a previous study of striatal DAT changes after glucose loading(11). This study was approved by the institutional review board of Pusan National University Hospital. An intravenous bolus injection of ^{18}F-FP-CIT was administered. The emission data were acquired over 90 mins with 50 frames of progressively increasing durations (15 s × 8 frames, 30 s × 16 frames, 60 s × 10 frames, 240 s × 10 frames, and 300 s × 6 frames) using the Siemens Biograph 40 Truepoint PET/CT (Siemens Healthcare, Knoxville, Tennessee, USA). The dynamic PET data were collected in the 3-dimensional mode, with 148 slices with image sizes of 256 × 256 and pixel sizes of 1.3364 × 1.3364 mm^2. These were reconstructed by filtered back projection using a Gaussian filter. For a volume-of-interest (VOI)-based analysis, an averaged image (0−10 min after injection) was created from dynamic PET frames and spatially normalized to ^{15}O-Water PET template in statistical parametric mapping 5 (Wellcome Trust Centre for Neuroimaging, United Kingdom). To extract time-activity curves (TACs) of VOIs from full dynamic PET scans, Oxford-GSK-Imanova striatal atlas (https://fsl.fmrib.ox.ac.uk/fsl) for ventral striatum (VST), caudate nucleus, putamen, and Automated Anatomical Labeling atlas (12) for middle frontal, orbitofrontal, anterior/middle/posterior cingulate, parietal, temporal cortices were applied. DAT availability, expressed in terms of binding potential (BP$_{ND}$), were measured by analyzing TACs with the simplified reference tissue method (13) with the cerebellum as a reference. Image analysis was done using pmod version 3.6 (PMOD Technologies LLC, Zurich, Switzerland).

mRNA data

Gene expression information investigated in this study was obtained from the freely available Allen Human Brain Atlas (www.brain-map.org). The details of the procedures regarding Allen Human Brain Atlas are reported previously by Hawrylycz et al(14). We downloaded mRNA values of the dopamine transporter to test for an association with the radiopharmaceutical ^{18}F-FP-CIT. The dataset of Allen Human Brain Atlas is derived from 6 healthy donors. Allen Human Brain Atlas contains gene expression profiles of DAT throughout the brain with 6 probes. We downloaded DAT mRNA expression data in log2-values for each sample. For VOI-based analysis, mRNA samples were averaged within VST, caudate nucleus, putamen, middle frontal, orbitofrontal, anterior/middle/posterior cingulate, parietal, temporal cortices.

Statistical analysis

Normality was assessed using the D'Agostino & Pearson normality test. Spearman correlation analysis was done for both 1) auto-correlation of BP$_{ND}$s of ^{18}F-FP-CIT PET, and DAT mRNA expressions, and 2) cross-correlation between BP$_{ND}$s of ^{18}F-FP-CIT PET, and DAT mRNA expressions to ensure validity and consistency. In addition, with mean

BP$_{ND}$s of ^{18}F-FP-CIT PET, and mean DAT mRNA expressions, Spearman correlation analysis was done for each VOIs. All analyses were conducted using Prism (v7.0d, GraphPad Software Inc, La Jolla, CA, USA).

Results

PET-derived BP$_{ND}$s from 35 subjects with mean age of 24.4 years (range 20 ~ 31 years), and DAT mRNA expressions of 6 probes from 6 subjects were included in this study. As the limited number of genomic samples was included, normal distribution of DAT mRNA expression could not be assumed. PET-derived BP$_{ND}$s from VST, caudate nucleus, putamen showed substantially higher levels than those from middle frontal, orbitofrontal, anterior/middle/posterior cingulate, parietal, temporal cortices. However, mRNA expression levels from probe 1 (A_24_P397435), 2 (CUST_942_PI417557136), 3 (CUST_941_PI417557136), 4 (CUST_588_PI416408490), 5 (CUST_927_PI417557136) were systemically lower than those from probe 6 (CUST_15799_PI416261804), which could be due to a lack of sensitivity of these probes (Fig. 1).

Auto-correlation

The auto-correlation of PET-derived BP$_{ND}$s for DAT was intermediate (mean ρ^2 = 0.6588) with ρ^2 ranging from 0.0811 to 1 (Fig. 2A). However, the auto-correlation of mRNA expression was weak across the probes with mean ρ^2 from 0.0889 to 0.2263 (mean ρ^2 probe 1, 0.1192; probe 2, 0.0889; probe 3, 0.1288; probe 4, 0.0640; probe 5, 0.1299; probe 6, 0.2263) (Fig. 2B, Table 1).

Table 1

Auto-correlation of BP$_{ND}$ of ^{18}F-FP-CIT PET and DAT mRNA expression

Variables	Auto-correlation (ρ^2)		
	Minimum	Maximum	Mean ± Standard deviation
^{18}F-FP-CIT PET BP$_{ND}$	0.0811	1	0.6588 ± 0.2092
DAT mRNA expression Probe 1	0.0003	0.4900	0.1192 ± 0.1550
Probe 2	0.0018	0.2909	0.0889 ± 0.0789
Probe 3	0.0025	0.3600	0.1288 ± 0.1099
Probe 4	0	0.3403	0.0640 ± 0.0968
Probe 5	0	0.5136	0.1299 ± 0.1528
Probe 6	0.0011	0.8711	0.2263 ± 0.2233

Cross-correlation

Between PET-derived BP$_{ND}$s, and mRNA expression, cross-correlations were weak with mean ρ^2 ranging from 0.0011 to 0.2220 (mean ρ^2 probe 1, 0.1412; probe 2, 0.0011; probe 3, 0.0828; probe 4, 0.1316; probe 5, 0.1382; probe 6, 0.2220) (Fig. 3, Table 2). In addition, after averaging PET-derived BP$_{ND}$s, and DAT mRNA expression levels according

to VOIs, cross-correlation was investigated for each probe. No significant association was observed except for probe 6 (ρ =0.6485, p = 0.0490) (Fig. 4).

Table 2
Cross-correlation of BP_{ND} of ^{18}F-FP-CIT PET and DAT mRNA expression

Variables	Auto-correlation (ρ^2)		
	Minimum	Maximum	Mean ± Standard deviation
Probe 1	0	0.7038	0.1412 ± 0.1723
Probe 2	0	0.5924	0.0011 ± 0.1957
Probe 3	0	0.5682	0.0828 ± 0.0928
Probe 4	0	0.5878	0.1316 ± 0.1559
Probe 5	0	0.5739	0.1382 ± 0.1386
Probe 6	0	0.8155	0.2220 ± 0.1629

Discussion

In this study, we examined the association between in-vivo protein expression of DAT from PET, and DAT mRNA expression from Allen Human Brain Atlas. Among 6 probes for DAT mRNA from Allen Human Brain Atlas database, 5 probes showed much lower expression levels of DAT mRNA than probe 6, probably due to a lack of sensitivity. Although mRNA expression of probe 6 showed the highest expression levels in each VOI, the auto-correlation between subjects (inter-subject correlation) was weak with mean ρ^2 of 0.2263, and cross-correlation with PET-derived BP_{ND}s was also weak with mean ρ^2 of 0.2220. However, mean mRNA expression of probe 6 showed the significant correlation with mean PET-derived BP_{ND}.

Previously, the predictive power of brain mRNA mappings of Allen Human Brain Atlas was investigated after comparison with PET-derived protein expressions including serotonin receptor (7–9), serotonin transporter (8, 9), opioid receptor (7), monoamine oxidase A (MAO-A) (9, 10). The association between mRNA expressions, and PET-derived protein expression were strong in serotonin receptors (7–9), or weak in opioid receptor (7). However, in studies of MAO-A mRNA expressions, inconsistent results were reported as weak correlation with ^{11}C-Harmine (9), and strong correlation with ^{11}C-Befloxatone (10).

Dopamine is a neurotransmitter that involves in reward-motivated behavior, and motor control (1). Brain dopamine neurotransmission is regulated by DAT, which drives reuptake of extracellular dopamine into presynaptic neurons (2). Dysfunction of DAT has been known to be linked to neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder (3), biopolar disorder (4), and alcoholism (5). In addition, DAT is a major target for various pharmacologically active drugs (2). From genomic maps such as Allen Human Brain Atlas, gene expression can be visualized across whole brain regions yielding insights into the relationship between structure and function of human brain (15), leading to help brain investigation, and drug development (7). Therefore, we investigated the association of DAT between protein expression measured by PET, and mRNA expression from Allen Human Brain Atlas. However, in this study, we observed relatively weak associations between DAT protein expressions, and DAT mRNA expressions regardless of probes. In addition, the correlation between mean PET-derived BP_{ND}s, and mean DAT mRNA expression

levels were marginally significant in 1 of 6 probes. Therefore, maps of the human mRNA transcription architecture from DNA microarray analysis of DAT might have limited predictive value, after comparison with PET-derived protein expression in this study. Previously, Rizzo et al. explained the possible mechanisms of the limited predictive role of mRNA mapping for protein expression (7). First, posttranscriptional mechanisms of splicing, or translational modifications might influence protein expression for each cell type (7, 16). In addition, mRNA expression is analyzed in the cytoplasm, while DAT protein is predominantly expressed presynaptically (9), which might affect the limited role of mRNA mapping for DAT.

There are several limitations in this study. First, the sample size of Allen Human Brain Atlas database is small with 6 donors. Second, although mRNA data of 6 probes were included across the regions, mRNA expression from 5 probes were systemically low, probably due to a lack of sensitivity of probes. In addition, the auto-correlation of mRNA expression between individuals was weak, probably due to interindividual variations. Also, DAT of both mRNA expressions, and PET-derived BP_{ND}s might be affected by the functional status of dopaminergic system, dopamine levels in the brain. Lastly, substantia nigra and ventral tegmental area might have a relevant role in the description of mRNA for DAT protein expression.

Conclusion

We observed weak associations between DAT mRNA expressions, and DAT protein expressions in human brains. Therefore, there might be a limited predictive power of DAT mRNA mappings for DAT protein expressions in humans.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Ethical permission for the study procedures was obtained from the Institutional Review Boards at Pusan National University Hospital. Subject consent has been obtained by Pusan National University Hospital. This article does not contain any studies with animals performed by any of the authors.

Consent for publication

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data are available to corresponding author of the manuscript upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

AUTHOR's CONTRIBUTIONS

Kyoungjune Pak; study design, write the manuscript

Seongho Seo; image analysis

Myung Jun Lee; image analysis, study design

Hyung-Jun Im: study design

Keunyoung Kim; image analysis

In Joo Kim; write the manuscript

References

1. Salamone JD, Correa M. Dopamine and food addiction: lexicon badly needed. Biol Psychiatry. 2013;73(9):e15-24.
2. Vaughan RA, Foster JD. Mechanisms of dopamine transporter regulation in normal and disease states. Trends Pharmacol Sci. 2013;34(9):489-96.
3. Roessner V, Sagvolden T, Dasbanerjee T, Middleton FA, Faraone SV, Walaas SI, et al. Methylphenidate normalizes elevated dopamine transporter densities in an animal model of the attention-deficit/hyperactivity disorder combined type, but not to the same extent in one of the attention-deficit/hyperactivity disorder inattentive type. Neuroscience. 2010;167(4):1183-91.
4. Mick E, Kim JW, Biederman J, Wozniak J, Wilens T, Spencer T, et al. Family based association study of pediatric bipolar disorder and the dopamine transporter gene (SLC6A3). Am J Med Genet B Neuropsychiatr Genet. 2008;147B(7):1182-5.
5. Du Y, Nie Y, Li Y, Wan YJ. The association between the SLC6A3 VNTR 9-repeat allele and alcoholism-a meta-analysis. Alcohol Clin Exp Res. 2011;35(9):1625-34.
6. Shen EH, Overly CC, Jones AR. The Allen Human Brain Atlas: comprehensive gene expression mapping of the human brain. Trends Neurosci. 2012;35(12):711-4.
7. Rizzo G, Veronese M, Heckemann RA, Selvaraj S, Howes OD, Hammers A, et al. The predictive power of brain mRNA mappings for in vivo protein density: a positron emission tomography correlation study. J Cereb Blood Flow Metab. 2014;34(5):827-35.
8. Beliveau V, Ganz M, Feng L, Ozenne B, Hojgaard L, Fisher PM, et al. A High-Resolution In Vivo Atlas of the Human Brain's Serotonin System. J Neurosci. 2017;37(1):120-8.
9. Komorowski A, James GM, Philippe C, Gryglewski G, Bauer A, Hienert M, et al. Association of Protein Distribution and Gene Expression Revealed by PET and Post-Mortem Quantification in the Serotonergic System of the Human Brain. Cereb Cortex. 2017;27(1):117-30.
10. Zanotti-Fregonara P, Leroy C, Rizzo G, Roumenov D, Trichard C, Martinot JL, et al. Imaging of monoamine oxidase-A in the human brain with [11C]befloxatone: quantification strategies and correlation with mRNA transcription maps. Nucl Med Commun. 2014;35(12):1254-61.
11. Pak K, Seo S, Kim K, Lee MJ, Shin MJ, Suh S, et al. Striatal Dopamine Transporter Changes after Glucose Loading in Humans. Diabetes Obes Metab. 2019.
12. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage. 2002;15(1):273-89.

13. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. Neuroimage. 1996;4(3 Pt 1):153-8.

14. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature. 2012;489(7416):391-9.

15. Sandberg R, Yasuda R, Pankratz DG, Carter TA, Del Rio JA, Wodicka L, et al. Regional and strain-specific gene expression mapping in the adult mouse brain. Proc Natl Acad Sci U S A. 2000;97(20):11038-43.

16. Cheng LC, Tavazoie M, Doetsch F. Stem cells: from epigenetics to microRNAs. Neuron. 2005;46(3):363-7.
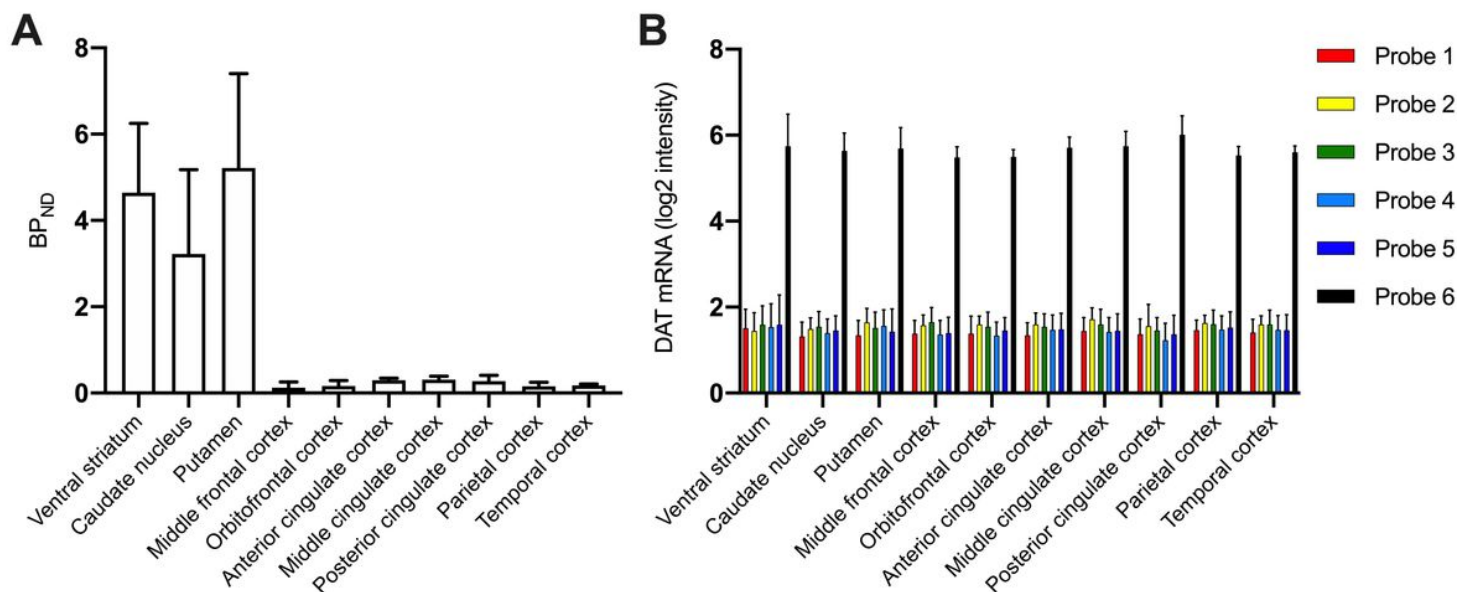
Figures

Figure 1

Mean and standard deviation of (A) PET-derived binding potential (BPND), and (B) mRNA expression
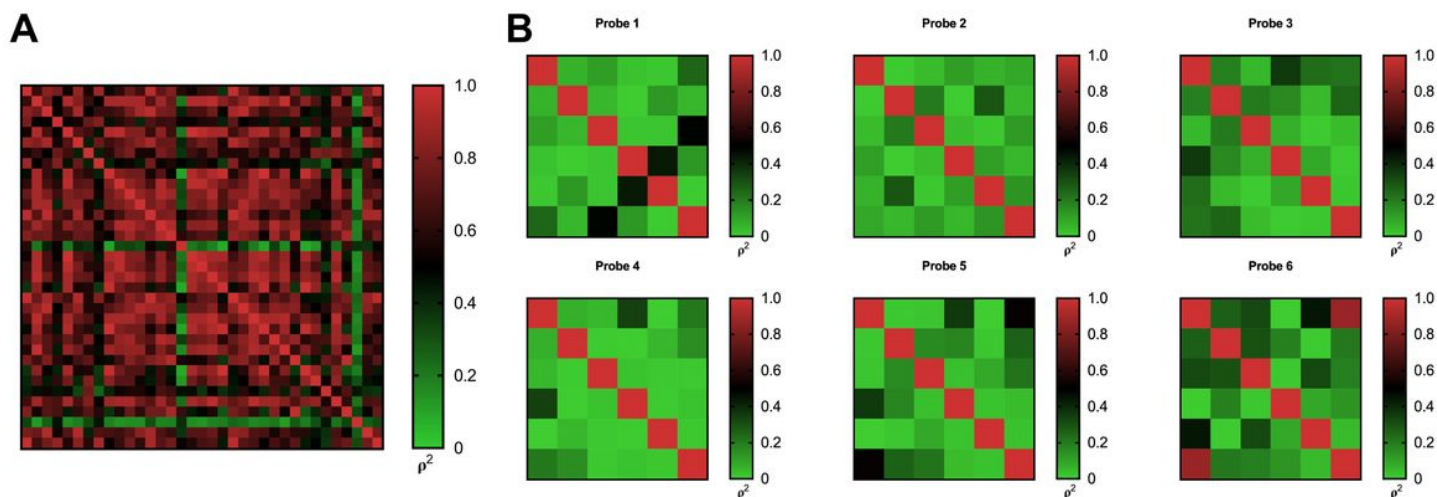

Figure 2

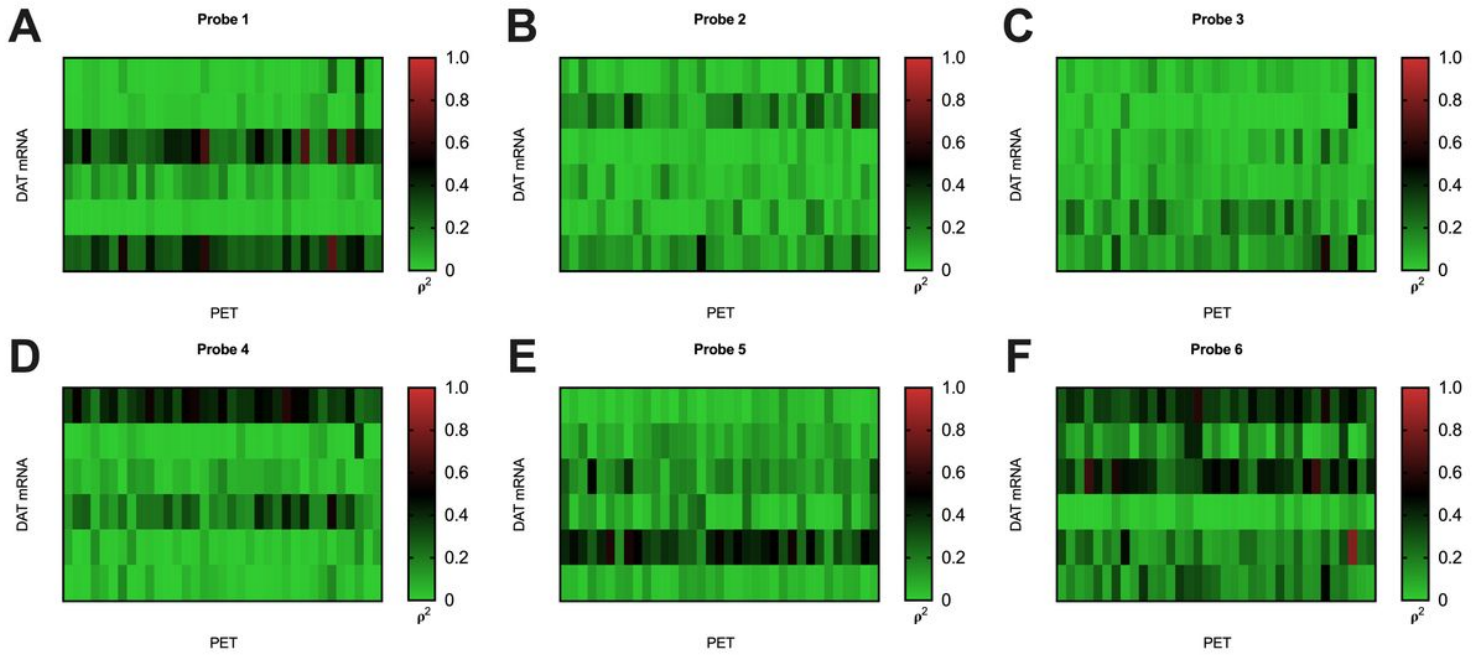Auto-correlations of (A) PET-derived binding potential (BPND), and (B) mRNA expression

Figure 3

Cross-correlations of PET-derived binding potential (BPND), and mRNA expression of 6 probes
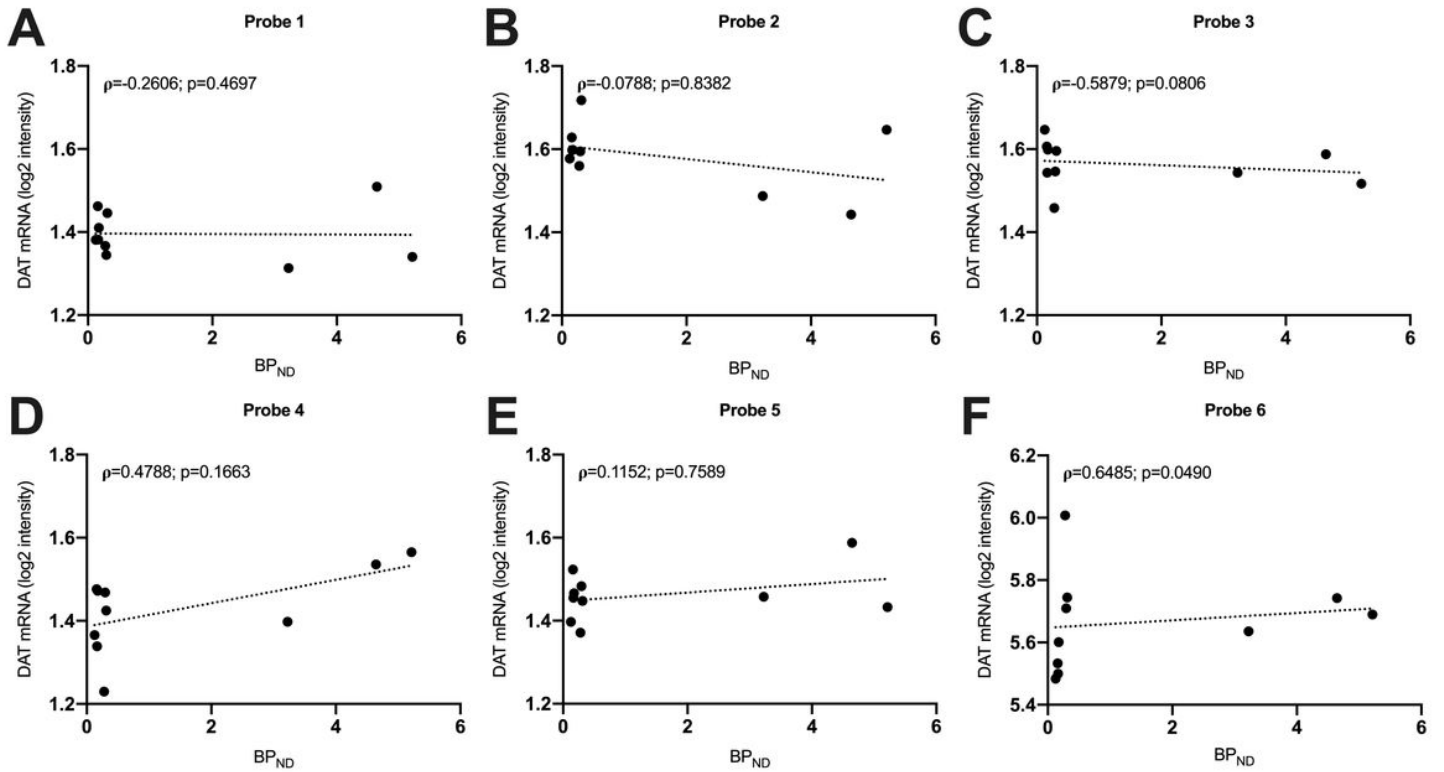

Figure 4

Cross-correlations of averaged PET-derived binding potential (BPND), and averaged mRNA expression of 6 probes