Supplementary Online Content


**eMethods.**

**eTable 1.** Demographic, Postmortem, and Clinical Characteristics of Human Subjects Used in This Study

**eTable 2.** Primer Design for qPCR and In Situ Hybridization

**eFigure 1.** Sampling Strategy for Grain Counting Analysis of NARP mRNA Expression

**eFigure 2.** Effect of Potentially Confounding Variables on NARP mRNA Expression Levels

**eFigure 3.** qPCR Determination for NARP mRNA Expression Levels in DLPFC Area 9 of Antipsychotic Medication–Exposed Monkeys

This supplementary material has been provided by the authors to give readers additional information about their work.
Human subjects

Brain specimens were obtained during autopsies conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, PA) following consent from the next-of-kin. An independent committee of experienced research clinicians made consensus DSM-IV–R diagnoses (American Psychiatric Association, 2000) for each subject, based on results of structured interviews conducted with family members and review of medical records, as described previously¹. The same approach was used to confirm the absence of a psychiatric diagnosis in healthy comparison subjects.

Tissue preparation

Frozen tissue blocks containing the middle portion of the right superior frontal sulcus were cut on a cryostat. The location of dorsolateral prefrontal cortex (DLPFC) area 9 was confirmed using Nissl-stained tissue sections for each subject¹. For quantitative PCR (qPCR) studies, gray matter from adjacent sections was separately collected into a tube containing TRIzol reagent (Invitrogen, Grand Island, NY) in a manner that ensured minimal white matter contamination and excellent RNA preservation². Total RNA for each subject was extracted and purified with RNeasy Mini Kit (Qiagen, Valencia, CA). For in situ hybridization (ISH), coronal cryostat sections (20 μm) from each subject were mounted on Superfrost Plus glass slides (Fisher Scientific, Hampton, NH) and stored at -80°C until processed. Three sections from each subject, evenly spaced at 320 μm, were matched within subject pairs and then used to assess NARP mRNA expression as described previously². Tissue samples from members of a subject pair were always processed together throughout all stages of an experiment.

Quantitative PCR (qPCR)

Total RNA was converted into complementary DNA (cDNA) for each subject using a High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA). The cycle threshold (CT) was determined for each transcript of interest using power SYBR Green master mix (Life Technologies) and ViiATM7 Real-Time PCR system (Life Technologies) as described previously³. All primer sets in this study showed ≥97% amplification efficiency in individual standard curve analyses and amplified a specific single product in dissociation curve analyses (eTable 2A). The difference in CT for each target transcript was calculated by subtracting the mean cycle threshold for the three internal reference transcripts (β-actin, cyclophilin A, and glyceraldehyde-3-phosphate dehydrogenase) from the mean cycle threshold of the target transcript. Because this difference in cycle threshold (ΔCT) represents the log₂-transformed expression ratio of each target transcript to the reference transcripts, the relative expression level of the target transcript is determined as 2^-ΔCT ⁴,⁵.

In situ hybridization (ISH)

Templates for the synthesis of riboprobes against human NARP were generated by PCR. The specific primer set amplified a 494 base-pair fragment corresponding to bases 1791-2284 of the human NARP gene (GenBank NM_002523) which was non-overlapping with the primer set used in the qPCR study. Nucleotide sequencing confirmed 100% homology of the amplified fragment to the reported NARP sequence (eTable 2B). Sense and antisense ³⁵S-riboprobes were generated by in vitro transcription.

One pair of sections from each subject pair was processed side by side in an ISH run. Following fixation with 4% paraformaldehyde in PBS solution, the sections were acetylated with 0.25% acetic anhydrate in 0.1 M triethanolamine/0.9% NaCl for 10 min and dehydrated with a graded alcohol series. The sections were then hybridized with ³⁵S-labeled riboprobes (1.0x10⁷ cpm/ml) in hybridization buffer at 56°C for 16 h. The hybridization buffer contained 50% formamide, 0.75 M NaCl, 20 mM 1,4-piperazine diethane sulfonic acid, pH 6.8, 10 mM EDTA, 10% dextran sulfate, 5x Denhardt’s solution (0.2 mg/ml Ficoll, 0.2 mg/ml Ficoll), 30 mg/ml OpenSSL.

© 2015 American Medical Association. All rights reserved.