***WEB SUPPLEMENT***

**Details of DNA Extraction and Genotyping Procedures in the SECCYD**

**Extraction**

Extraction for all polymorphisms was based on adaptations to Freeman et al. (2003). Specifically, buccal mucosa cells were collected with cotton swabs by the subject. The swabs were placed in 15-ml centrifuge tubes containing 2.5 ml of lysis buffer. The tubes were incubated in a water bath at 65 °C for 2 hr to activate the proteinase K. After incubation the tubes were centrifuged at 300 × *g* for 4 min and the supernatant added to 4 ml of isopropanol. Tubes were centrifuged again for 30 min. The supernatant was poured off, the pellet was dried, and 1 ml of lysis buffer without proteinase K was added. Pellets were resuspended by shaking overnight. The liquid was transferred to a 1.5-ml microfuge tube and 200 µl of an organic deproteinization reagent were added to each tube. The tubes were capped and shaken vigorously by hand. The denatured debris and remaining organic mix were then centrifuged at 5000 × *g* for 10 min. Supernatant from the tube was transferred to a fresh 1.5-ml tube and 800 µl of isopropanol was added and mixed gently for approximately 1 min. The DNA was collected by centrifugation at 5000 × *g* for 10 min. The pellets were dried and washed with 1 ml ethanol 70% (v/v) by centrifugation at 5000 × *g* for 10 min. The ethanol wash was discarded, the tubes were inverted, and the pellets were dried for 60 min. The DNA was resuspended in 250 µl of Tris EDTA (TE) buffer (10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0) by rotation in an incubator at 37 °C. The DNA was quantified by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer. Samples were aliquoted into storage vials and placed in a –80 °C freezer.

**Genotyping**

**Dopamine Receptor D2 (*DRD2*) rs1800497 Single Nucleotide Polymorphisms (SNP)**. In order to genotype *DRD2*, Taqman SNP Genotyping Assays were performed using an Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) protocol. Forty nanograms of DNA were combined in a volume of 5 µl with 2X Universal PCR Mix (Applied Biosystems) and 1/20 the volume of the Taqman SNP assay in a 384 well plate. A preread was performed and then polymerase chain reaction (PCR) as follows: a 10 min hold at 95 °C, followed by 40 to 45 cycles of 15 s at 92 °C, and then 1 min at 60 °C in a 7900HT PCR system. After amplification, a postread was performed to analyze the data. Automatic and manual calls were made. Reliability genotyping for this SNP was conducted on an open array (see description below).

**Catechol-*O*-methyltransferase (*COMT*) rs4680 SNP**. For *COMT*, Taqman SNP Genotyping Assays were performed using an Allelic Discrimination Assay (Applied Biosystems) protocol. Forty ng of DNA were combined in a volume of 5 µl with 2X Universal PCR Mix (Applied Biosystems) and 1/20 the volume of the Taqman SNP assay in a 384 well plate. A preread was performed and then PCR as follows: a 10 min hold at 95 °C, followed by 40 to 45 cycles of 15 s at 92 °C and then 1 min at 60 °C in a 7900HT PCR system. After amplification, a postread was performed to analyze the data. Automatic and manual calls were made. Reliability genotyping was conducted on an open array (see description below).

**Brain-derived neurotrophic factor (*BDNF*) rs6265, tryptophan hydroxylase 1 (*TPH1)* rs1800532, serotonin-2A receptor (*HTR2A*) rs6313, opioid receptor μ1 (*OPRM1*) rs1799971, corticotropin releasing hormone receptor 1 (*CRHR1*) rs242924, and *CRHR1* rs7209436 SNP genotyping**. These SNPs (including relevant reliability genotyping) were genotyped on an Open Array. Specifically, SNPs were determined using the Open Array© Real-Time PCR system (Applied Biosystems) as in User Guides PN 4458837 Rev. A and PN 4458840A. Briefly, genomic DNA was added to 96 well plates at the recommended concentration and then transferred to 384 well plates along with the TaqMan OpenArray Gene Expression Master Mix. The 384 well plates and OpenArray© slides were placed in an Open Array AutoLoader (Applied Biosystems) that automatically transferred the solution from the plate to the slide. The slide was placed in the Genotyping Case and then in an Applied Biosystems GeneAmp PCR system 9700 thermal cycler (Applied Biosystems). The Open Array Run Method was 93 °C for 10 m followed by 60 cycles of 95 °C for 45 min, 94 °C for 13 min, and 53 °C for 2 min, 14 s. A final step of 25 °C for 2 min was run before setting the module to 4 °C. A file composed of sample names was prepared and uploaded into the Open Array Image software along with the serial number of the slide. After cycling, the slide was placed in the Open Array NT Imager and imaged. OpenArray SNP Genotyping Analysis Software v 1.0.3 was used to analyze the data.

**Dopamine Receptor D4 (*DRD4*) Variable Number Tandem Repeat (VNTR)**. The assay for genotyping the *DRD4* VNTR was based on methods developed Sander et al. (1997) and modified by Anchordoquy, McGeary, Liu, Krauter, and Smolen (2003). The Genomics Core Facility modified it further as the following: 1X Taq Gold Buffer, 2.25 m*M* final concentration of MgCl2, 10% DMSO, 0.2 m*M* dNTPs, 0.1 m*M* deazo GTP, 0.75 µ*M* primers, 40 ng of DNA, and 1 U of Taq Gold (Applied Biosystems) in a volume of 12 µl. The primer sequences were forward, 5'-6-FAM-GCGAC TACGTGGTCTACTCG-3' and reverse, 5'-AGGACCCTCATGGCCTTG-3'. The amplification procedure was as described by Anchordoquy et al. One µl was removed and placed in a 96 well plate and 10 µl of formamide containing LIZ-500 standard (Applied Biosystems). The plate was run using a fragment analysis protocol in the 3730XL DNA Analyzer (Applied Biosystems). Fragments were analyzed using Genemapper software (Applied Biosystems) with PCR products of 379, 427, 475 (43), 523, 571, 619 (73), 667, 715, 763, and 811 base pairs (bp). Reliability genotyping was also conducted as described immediately above.

**Serotonin Transporter (*5-HTT*) VNTR**. The assay for *5-HTT* was a modification of the method of Lesch et al. (1996) and Anchordoquy et al. (2003). The Genomics Core Facility modified it further as the following: 1X Taq Gold Buffer, 1.8 m*M* final concentration of MgCl2, 10% DMSO, 0.2 m*M* dNTPs, 0.1 m*M* deazo GTP, 0.6 µ*M* primers, 40 ng of DNA, and 1 U of Taq Gold (Applied Biosystems) in a volume of 15 µl. The primer sequences were forward, 5'-VIC-GGCGTTGCCGCTCTGAATGC-3', and reverse, 5'-GAGGGACTGAGCTGGACAACCAC-3'. The same amplification protocol as used for *DRD4* VNTR was used for the *5-HTT* VNTR. One microliter was removed and placed in a 96 well plate and 10 µl of formamide containing LIZ-500 standard (Applied Biosystems). The plate was run using a fragment analysis protocol in the 3730XL DNA Analyzer (Applied Biosystems). Fragments were analyzed using Genemapper software (Applied Biosystems) with PCR products of 484 or 528 bp. Reliability genotyping was also conducted as described immediately above.

 **Dopamine Transporter 1 (*DAT1*) VNTR.** The assay was a modification of the method of Vandenbergh et al. (1992; Anchordoquy et al. 2003). The Genomics Core Facility modified it further as the following: 1X Taq Universal Master Mix (Applied Biosystems), 0.75 uM primers, and 40 ng of DNA in a volume of 15 µl. The primer sequences were forward, 5'-VIC- TGTGGTGTAGGGAACGGCCTGAG-3', and reverse, 5'-CTTCCTGGAGGTCACGGCTCAAGG-3'. The amplification protocol was 95 oC for 5 min followed by 40 cycles of 95 oC for 30 s, 60 oC for 30 s, and 72 oC for 1 min. An extension step of 72 oC followed for 5 min. One µl was removed and placed in a 96 well plate and 10 µl of formamide containing LIZ-500 standard (Applied Biosystems). The plate was run using a fragment analysis protocol in the 3730XL DNA Analyzer (Applied Biosystems). Fragments were analyzed using Genemapper software (Applied Biosystems) with PCR products.

 **Monoamine oxidase A (*MAOA*) VNTR.** The assay was a modification of the method of Sabol et al. (1998). The Genomics Core Facility modified it further as the following: 1X Taq Universal Master Mix (Applied Biosystems), 0.75 µ*M* primers, and 40 ng of DNA in a volume of 15 µl. The primer sequences were forward, 5'-NED-ACA GCC TGA CCG TGG AGA AG-3' and reverse, 5'-GAA CGG ACG CTC CAT TCG GA-3'. The amplification protocol was 95 oC for 5 min followed by 40 cycles of 95 oC for 30 s, 60 oC for 30 s, and 72 oC for 1 min. An extension step of 72 oC followed for 5 min. One µl was removed and placed in a 96 well plate and 10 µl of formamide containing LIZ-500 standard (Applied Biosystems). The plate was run using a fragment analysis protocol in the 3730XL DNA Analyzer (Applied Biosystems). Fragments were analyzed using Genemapper software (Applied Biosystems) with PCR products of 258, 288, 318, and 348 bp.

**References for Web Supplement**

Anchordoquy, H. C., McGeary, C., Liu, L., Krauter, K. S., & Smolen, A. (2003). Genotyping of three candidate genes after whole-genome preamplification of DNA collected from buccal cells. *Behavioral Genetics, 33*, 73–78.

Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J., & Craig, I. W. (2003). DNA from buccal swabs recruited by mail: Evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. *Behavioral Genetics, 33*, 67–72.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274*, 1527–1531.

Sabol, S. Z., Hu, S., & Hamer, D. (1998) A functional polymorphism in the monoamine oxidase A gene promoter. *Human Genetics*, *103*, 273–279.

Sander, T., Harms, H., Dufeu, P., Kuhn, S., Rommelspacher, H., & Schmidt, L. G. (1997). Dopamine D4 receptor exon III alleles and variation of novelty seeking in alcoholics. *American Journal of Medical Genetics, 74*, 483-487.

Vandenbergh, D. J., Perisco, A. M., Hawkins, A. L., Griffin, C. A., Li X., Jabs, E. W., et al. (1992). Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. *Genomics, 14*, 1104–1106.