Biological Effects of *COMT* Haplotypes and Psychosis Risk in 22q11.2 Deletion Syndrome

Doron Gothelf, Amanda J. Law, Amos Frisch, Jingshan Chen, Omer Zarchi, Elena Michaelovsky, Renee Ren-Patterson, Barbara K. Lipska, Miri Carmel, Bhaskar Kolachana, Abraham Weizman, and Daniel R. Weinberger

Background: 22q11.2 deletion syndrome (22q11.2DS) is the most common genetic syndrome associated with schizophrenia. The catechol-*O*-methyltransferase (*COMT*) gene is located in the obligatory deletion region, and possible associations between *COMT* variants and neuropsychiatric manifestations in 22q11.2DS have been reported. The purpose of the current study was to evaluate the effect of *COMT* hemizygosity and molecular haplotypes on gene expression and enzyme activity and its association with psychotic symptoms in 22q11.2DS.

Methods: Lymphoblast samples were drawn from 53 individuals with 22q11.2DS and 16 typically developing control subjects. We measured COMT messenger (m)RNA and protein expression and enzyme activity using standard procedures. The presence of a psychotic disorder and cognitive deficits were also evaluated using structured testing.

Results: There was an approximately 50% reduction in COMT mRNA, protein, and enzyme activity levels in 22q11.2DS samples. Haplotype analysis revealed clear phenotypic differences between various Val-containing haplotypes on *COMT*-3′ untranslated region extended mRNA, soluble COMT and membrane-bound proteins, and enzyme activity. The G variant of rs165599, a 3′ untranslated region single nucleotide polymorphism, was associated with low levels of COMT expression and with the presence of psychosis and lower performance IQ scores in our 22q11.2DS sample. Finally, we demonstrate that the *COMT* rs74745580 "T" mutation is associated with absent soluble COMT expression and very low COMT activity in two 22q11.2DS individuals.

Conclusions: Our findings confirm a robust effect of *COMT* hemizygosity on COMT activity and show complex interactions of variants within the *COMT* gene that influence COMT biology and confound conclusions based on associations with the Val158Met genotype alone.

Key Words: *COMT*, DiGeorge, gene expression, haplotype, psychosis, velocardiofacial syndrome

Occurring in at least 1 of 4000 live births, 22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial syndrome and DiGeorge syndrome, is a multisystem congenital anomaly disorder caused by a microdeletion of one copy of chromosome 22q11.2 (1). The syndrome is associated with high rates of neuropsychiatric morbidity and cognitive deficits (2–4). The average IQ in 22q11.2DS is 75 (within the range of borderline intellectual function) (5), and 25% to 33% of individuals develop schizophrenia (2,6,7). Studies of patients diagnosed as having schizophrenia show that as many as .3% to 2% of them carry the 22q11.2 deletion (8), suggesting that the 22q11.2 region includes a gene or genes that have an impact on risk for schizophrenia.

Of the 28 genes within the 22q11.2DS-critical region, several have been independently associated with non-22q11.2DS schizophrenia [see Karayiorgou *et al.* for review (9)], including catechol-*O*-methyltransferase (*COMT*) (10–12). The *COMT* gene has been extensively studied in terms of its association with psychiatric disease and cognitive function (10,13). *COMT* contains a common functional polymorphism, Val158Met (rs4680), which affects enzyme activity through differential protein thermostability (14–16) and has been shown to affect prefrontal cortical physiology, working memory, and emotional regulation in humans (10,17,18). These results are consistent with the major role of COMT in modulating dopamine flux in prefrontal and hippocampal cortices (19,20) and with the importance of dopamine in tuning cortical information processing. Although *COMT* has been a popular candidate gene for psychiatric illness, most of the studies on *COMT* and its clinical associations, which have focused on the Val158Met variant, have failed to yield compelling results (21). One possible explanation for the inconsistencies in the literature is that the effect of the Val158Met variant on COMT function risk for schizophrenia is modulated by other functional variants in *COMT* (22–25).

Another possible explanation for inconsistencies involves the role of epigenetic mechanisms that regulate COMT expression. For example, methylation of CpG sites in the *COMT* promoter region have been shown to affect COMT expression in the brain and to be associated with risk for schizophrenia and prefrontal cognitive function (26,27). Methylation also occurs at the *COMT* Val158Met site and has been demonstrated

From the Behavioral Neurogenetics Center (DG, OZ), Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Israel; Sackler Faculty of Medicine (DG, AF, OZ, AW), Tel Aviv University, Tel Aviv, Israel; Departments of Psychiatry and Cell and Developmental Biology (AJL), School of Medicine, University of Colorado, Aurora, Colorado; Clinical Brain Disorders Branch (AJL, JC, RR-P, BKL, BK, DRW), Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland; The Felsenstein Medical Research Center (AF, EM, MC, AW), Petah Tiqwa, Israel; and Lieber Institute for Brain Development (DRW), Johns Hopkins University Medical Campus, and Departments of Psychiatry, Neurology, and Neuroscience and the McKusick-Nathans Institute of Genetic Medicine (DRW), Johns Hopkins University School of Medicine, Baltimore, Maryland.

Authors DG and AJL contributed equally to the work.

Address correspondence to Daniel R. Weinberger, M.D., Lieber Institute for Brain Development, Johns Hopkins University School of Medicine, 855 Wolfe Street, Baltimore, MD 21205; E-mail: drweinberger@libd.org.

Received Mar 25, 2013; revised and accepted Jul 22, 2013.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.07.021

to modulate activity of the Val allele (cytosine) but not the Met allele (adenine) (27).

The association between the Val158Met genotype on the intact chromosome and cognitive/psychiatric phenotypes has been investigated in 22q11.2DS (6,28–31). Because the Met allele translates into a less heat-stable protein, it is assumed that individuals with 22q11.2DS who have a single copy of the Met allele have markedly low COMT activity and especially high levels of cortical dopamine, which would adversely influence cortical function. Some studies have supported this assumption (6,29,31,32): compared with 22q11.2DS adults carrying the *COMT* Val allele (high-activity allele), those carrying the Met allele (low-activity allele) tend to have increased risk for psychotic disorders (33) and other neuropsychiatric syndromes (30) and have more severe cognitive deficits (6,29,31). Other studies, however, have not found an association between *COMT* Val158Met genotype and psychosis (7,34) or cognitive functioning (28).

In this report, we address the possibility that other variants in *COMT* modulate the Val/Met effect. The variants selected for this study include those previously associated with functional effects: 1) a single nucleotide polymorphism (SNP; rs2075507) located in the P2 promoter region of *COMT* (and also mapped to neighboring *TXNRD2*) was shown to affect the transcription of COMT and consequently the protein levels and enzyme activity in human brain (14). 2) rs4633 and rs4818 are synonymous SNPs located in coding exons of *COMT*. These two SNPs affect messenger (m)RNA secondary structure, which has an impact on the efficiency of COMT protein translation and thus enzyme activity (25). 3) SNPs within the 3′ untranslated region (3′UTR) of *COMT*-rs2828146 and -rs165599. rs165599 has been associated with COMT mRNA levels in normal human brain presumably by altering microRNA-mediated RNA processing (22,23). 4) Two SNPs (rs2073748 and rs2240717) from the *ARVCF* gene, a gene that is adjacent to *COMT* and shares 3′sequence with the long 3′UTR of *COMT*. These two nonsynonymous SNPs cause amino-acid changes in the *ARVCF* sequence (24) (see Table S1 in Supplement 1).

Given that individuals with 22q11.2DS are hemizygous for genes in the microdeletion region, the disorder represents a unique human genetic model to study the biological effects of molecular haplotypes that have an impact on gene function. Our specific hypotheses were as follows: 1) individuals with 22q11.2DS would have approximately 50% less *COMT* gene expression, protein levels, and enzyme activity compared with typically developing control subjects with two copies of *COMT*; 2) there would be significant effects of SNPs and haplotypes, previously associated with COMT biology, prefrontal cognitive functioning, and/or schizophrenia (14,22,24,25,35,36), on COMT mRNA and protein levels and enzyme activity, as well as on the risk for psychosis and cognitive deficits associated with 22q11.2DS; and 3) combinations of alleles of functional SNPs in *COMT* on the intact chromosome would alter the associations with the Val/Met variant alone.

Methods and Materials

Human Study Samples

Individuals with 22q11.2DS were recruited from the Behavioral Neurogenetics Center at a large tertiary referral center in Israel, and control subjects were taken from samples of European ancestry at the National Institute of Mental Health (see Methods in Supplement 1) (10). The study protocol was approved by the Institutional Review Board of Rabin Medical Center and the National Institute of Mental Health Institutional Review Board, and informed consent was obtained from all participants or their parents or guardians. Psychiatric and IQ assessments were conducted when the biological samples were collected as part of our 22q11.2DS longitudinal study.

B Lymphoblast Culture, RNA Extraction, and Genotyping

Transformed lymphoblast cell lines and RNA extraction was performed according to standard protocols as described in Methods in Supplement 1. Eight SNPs previously identified as either functional or associated with clinical phenotypes were examined (11,14,24,35) (see Figure 1 and Table S1 in Supplement 1). This eight-SNP haplotype was previously associated with attention-deficit/hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD), in a sample consisting of 28 of the current 53 subjects, when they were on average 6.6 ± 2.5 years younger, whereby A-C-G-G-C-G-C-T was associated with lower prevalence of ADHD and OCD (24).

Quantitative Real-Time Polymerase Chain Reaction, Western Blotting, and Sequencing

COMT mRNA expression levels were measured by real-time quantitative reverse transcriptase polymerase chain reaction as described in Methods in Supplement 1. Two COMT mRNAs assays were used in our study, one that detects all COMT transcripts (referred to as "COMT-common") and another that measures COMT transcripts containing an extended 3′UTR referred to as ("COMT-extended"; see Methods in Supplement 1). Protein immunoreactivity was measured by Western blotting. The Sanger method was used for sequencing the two isoforms of COMT cDNA—the membrane-bound COMT (MB-COMT, 28 kDa) and soluble COMT (S-COMT, 25 kDa; see Methods in Supplement 1).

COMT Enzyme Activity Assay

Enzyme activity of MB-COMT and S-COMT was assayed based on the organic solvent extraction method that separates the radioactive product, the methylated catechol, and the free radioactive coenzyme [3H]AdoMet (14,37). This is described in detail in Methods in Supplement 1.

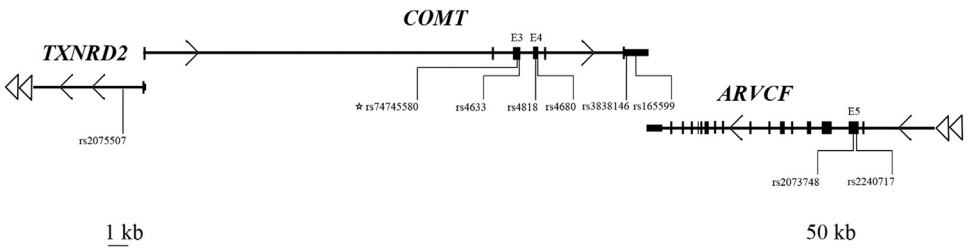

Figure 1. Diagram of the *TXNRD2*, *COMT*, and *ARVCF* genes showing locations of genotyped single nucleotide polymorphisms.