NIH Public Access
Author Manuscript
Biol Psychiatry. Author manuscript; available in PMC 2015 March 01.

Published in final edited form as:
Biol Psychiatry. 2014 March 1; 75(5): 406–413. doi:10.1016/j.biopsych.2013.07.021.

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

Abstract

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 controls. We measured COMT mRNA 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 a ~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′UTR extended mRNA, S-COMT and MB proteins and enzyme activity. The G variant of rs165599, a 3′UTR SNP, 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 S-COMT expression and very low COMT activity in two 22q11.2DS individuals.

© 2013 Society of Biological Psychiatry. Published by Elsevier Inc. All rights reserved.

Correspondence: Daniel R. Weinberger, M.D. Lieber Institute for Brain Development, Johns Hopkins University School of Medicine 855 Wolfe Street, Baltimore, Maryland 21205; drweinberger@libd.org.
[*]authors contributed equally to the work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Financial Disclosures
The authors report no biomedical financial interests or potential conflicts of interest.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

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.

Keywords

velocardiofacial syndrome; DiGeorge; haplotype; psychosis; COMT; gene expression

Introduction

22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial syndrome and DiGeorge syndrome, is a multi-system congenital anomaly disorder caused by a microdeletion of one copy of chromosome 22q11.2. 22q11.2DS, occurring in at least 1 of 4,000 live births (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 0.3%–2% of them carry the 22q11.2 deletion (8), suggesting that the 22q11.2 region includes a gene or genes that impact risk for schizophrenia.

Of the 28 genes within the 22q11.2DS-critical region, several have been independently associated with non-22q11.2DS schizophrenia (see (9) for review), 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 impacts 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 DA 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 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). Yet

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

other studies have not found an association between *COMT* Val158Met genotype and psychosis (7, 34) or cognitive functioning (28).

In the present 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: (i) A SNP (rs2075507) located in the P2 promoter region of *COMT* (and also mapped to neighboring *TXNRD2*) which was shown to affect the transcription of COMT and consequently the protein levels and enzyme activity in human brain (14). (ii) rs4633 and rs4818 are synonymous SNPs located in coding exons of *COMT*. These two SNPs affect mRNA secondary structure which impacts the efficiency of COMT protein translation and thus enzyme activity (25). (iii) 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). (iv) Two SNPs (rs2073748 and rs2240717) from the *ARVCF* gene, a gene that is adjacent to *COMT* and that shares 3′UTR sequence with the long 3′UTR of *COMT*. These two non-synonymous SNPs cause amino-acid changes in the *ARVCF* sequence (24) (see Supplement: Table S1).

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 impact on gene function. Our specific hypotheses were: (i) individuals with 22q11.2DS would have ~50% less *COMT* gene expression, protein levels and enzyme activity compared to typically developing (TD) controls with two copies of *COMT*; (ii) 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 (iii) 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

22q11.2DS individuals were recruited from the Behavioral Neurogenetics Center at a large tertiary referral center in Israel and controls were taken from samples of European ancestry at the NIMH (See Supplementary Methods) (10). The study protocol was approved by the Institutional Review Board of Rabin Medical Center and the NIMH Institutional Review Board, and informed consent was obtained from all participants and/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 Supplementary Methods. Eight SNPs previously identified as either functional or associated with clinical phenotypes were examined (11, 14, 24, 35) (See Figure 1, Supplementary Methods and Table S1). This 8 SNP haplotype was previously associated with 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).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Quantitative Real-Time PCR, Western Blotting and Sequencing

COMT mRNA expression levels were measured by real-time quantitative RT-PCR as described in Supplementary Methods. Two COMT mRNAs assays were used in our study, one that detects all COMT transcripts referred to as “COMT-common”, and the other that measures COMT transcripts containing an extended 3-UTR referred to as “COMT-extended” (see Supplementary Methods). 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, 28kDa) and soluble COMT S-COMT (25kDa) (see Supplementary Methods).

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 the Supplementary Methods.

Neuropsychiatric Assessment

22q11.2DS subjects were evaluated for the presence of psychotic disorders by a trained psychiatrist using the K-SADS (children and adolescents) or SCID (adults) and age-appropriate Wechsler Intelligence tests (2).

Statistical Analysis

Comparisons between 22q11.2DS and healthy comparison subjects and within 22q11.2DS were made using univariate analysis of variance (ANOVA) with age and sex included as potential covariates in all analyses. Correlations were determined with the Pearson’s Test and the effect of *COMT* genotypes on the presence of a psychotic disorder in 22q11.2DS was assessed using Pearson’s chi-square test.

Results

22q11.2DS vs. Normal Controls

To compare main effects of hemizygosity, we selected a subset of 20 adult individuals with 22q11.2DS that were age and sex matched to 16 healthy adult comparison subjects (28.8 ± 6.4 years vs. 31.4 ± 8.4 years, $p = .32$ and male/female ratio of 10/10 vs. 7/9, $p = .49$, respectively). As expected there were significant, robust differences between 22q11.2DS and controls in all COMT products including COMT-extended and COMT-common mRNAs, S-COMT and MB-COMT protein levels and COMT enzyme activity (all $p < .0001$) (Figure 2).

There was a significant age effect on S-COMT ($F = 11.6$ $p < .005$), and MB-COMT levels ($F = 12.7$, $p = .001$) and enzyme activity ($F = 15.5$, $p < .0001$). Additionally, an effect of sex was observed for MB-COMT levels, which was higher in male subjects ($F = 5.6$, $p = .03$).

SNP and Haplotype Analyses within the 22q11.2DS Group

Fifty-three individuals with 22q11.2DS were included in the within-group analyses. They consisted of 31 males and 22 females with a mean age of 19.5 ± 8.9 years.

Effects of COMT rs4680 Met/Val Genotype in 22q11.2DS on COMT Biology— Twenty-nine 22q11.2DS subjects were *COMT* Met allele carriers and 24 subjects were Val allele carriers. These two subgroups were similar in age and sex distributions (18.2 vs. 21.0 years; $p = .27$; M/F 18/11 vs. 13/11; $p = .38$, Met and Val, respectively). In 22q11.2DS,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

genotype had a major effect on COMT-common mRNA expression (F(1, 51) = 4.7, p = .03) and S-COMT protein levels (F(1, 49) = 13.1; p = .001). The *COMT* Met carriers exhibited lower levels of COMT-common mRNA and S-COMT levels than *COMT Val* carriers (Figure 3A). No effect of Val158Met genotype was observed on COMT-3′UTR extended mRNA expression levels (p = .26), MB-COMT protein levels (p > .70), and COMT enzyme activity (p = .16, Figure 3B).

Effects of a COMT 3-Marker Haplotype (rs2075507-rs4680-rs165599) in 22q11.2DS on COMT Biology—The three-marker haplotype, consisting of rs2075507 in the *COMT* promoter (also in *TXNRD2)*. Val158Met, and rs165599 had been found in a prior study to influence frontal lobe physiology more strongly than the effect of Val158Met genotype alone (36). The same haplotype was also associated with both OCD and ADHD diagnoses in a subgroup of this same sample when they were younger (33). We tested this haplotype in our current analyses. A highly significant effect of the 3-marker haplotype was observed on S-COMT levels (F(4, 46) = 6.2; p < .0001; Figure 4A). Post hoc analysis revealed that the COMT AGA haplotype had significantly higher S-COMT levels compared to the other 4 haplotypes (Figure 4A and Table 1). Similar effects of haplotypes (although less robust) were observed for MB-COMT levels (Table 1). COMT enzyme activity also significantly differed between haplotypes (F(4,48) = 2.4; p < .05), with the COMT AGA haplotype showing significantly higher enzyme activity levels compared to the AGG and GAA haplotypes (Figure 4B and Table 1), consistent with observed COMT protein level differences.

A significant effect of the 3-marker haplotype was also found in COMT-extended mRNA expression (F(4,48) = 2.8; p = .03; Table 1). Post hoc analysis revealed that the COMT AGA and AAA haplotypes exhibited higher COMT-extended mRNA expression levels compared to the AGG haplotype (Table 1). Interestingly, the Val- and Met-containing chromosomes (AGA and AAA, respectively) both had the A allele at rs165599, a SNP which resides in the extended 3′UTR and has been shown to have allele- specific association with levels of COMT mRNA in normal human brain (22). Single point analysis of rs165599 confirmed a specific and significant association with COMT-extended mRNA levels (F(1,52) = 7.85; p = .007; A = 0.60 *SD* = 0.19; G = 0.47, *SD* = 0.08), whereby the A allele was associated with higher mRNA levels than the G allele at this locus. This effect of rs165599 appears to be specific for the extended 3′UTR as it was not seen with the “common” *COMT* 3′UTR transcript.

Effects of a COMT 8-Marker Haplotype (rs2075507-rs4633-rs4818-rs4680-rs3838146-rs165599-rs2073748-rs2240717) in 22q11.2DS on COMT—To analyze maximum genetic diversity in our sample, we explored the effects of molecular haplotypes consisting of alleles at all 8 SNP loci. ANOVA revealed a significant effect of haplotype on S-COMT (F(5,38) = 4.5; p < .003) and MB-COMT protein levels (F(5,40) = 3.0; p < .03), with HAP1 showing significantly higher protein levels than the other HAPs (Supplement: Table S2). There was also a marginally significant effect of haplotype on COMT enzyme activity (F(5,40) = 2.4, p = .09) consistent with the COMT protein findings (Supplement: Table S2).

COMT rs74745580 Influences S-COMT Protein Levels and COMT Enzyme Activity

Two patients (I13 and I50) in our sample showed very low COMT enzyme activity (Figure 5A) and no detectable S-COMT levels (Figure 5B&C). To investigate this further, we cloned and sequenced COMT cDNA from the lymphoblast cells of these two patients. The sequence analysis revealed a rare single nucleotide variation ‘T’ located 34 nucleotides upstream of the start codon for S-COMT. This is a synonymous mutation that does not

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

change the amino acid (Asn) codon for MB-COMT. The sequence corresponds to a rare variant, rs74745580, identified in the 1000Genomes project (http://www.1000genomes.org). Our results suggest that the 'T' allele at rs74745580 could reduce translation of the short transcript from the human COMT gene, which encodes S-COMT.

Correlations Between COMT mRNA, Protein and Enzyme Activity Levels

There was a strong positive correlation between S-COMT levels and COMT enzyme activity ($r=0.72$, $p<0.0001$) for the whole sample and also for the individual 22q11.2DS and control groups. There were no significant correlations between MB-COMT levels and COMT enzyme activity.

Association between COMT SNPs, Psychosis, and Cognition in 22q11.2DS

Fourteen of the 53 22q1 1.2DS subjects (26.4%, 9 females and 5 males) fulfilled DSM-IV-TR criteria for a psychotic disorder: schizophrenia (N=8), psychotic disorder NOS (N=3), psychotic depression (N=2), and schizophreniform disorder (N=1). There were no significant differences in the rate of psychosis between Met and Val carriers. In contrast, rs165599 was associated with the presence of psychosis [G allele = 9 of 14 (64%) in the psychotic group vs. 12 of 39 (31%) in the non-psychotic group, *LRT p = .029; Chi-square p = .028*]. The G allele of rs165599 was also associated with lower performance IQ (PIQ) scores (G vs. A allele, 68.3 ± 9.0 vs. 77.0 ± 10.6, respectively, $t = 3.0$, $p = .005$). Neither rates of psychotic disorders nor IQ scores were significantly associated with the 3-marker or 8-marker haplotypes.

Discussion

The principal findings of this study are: (i) 22q11.2DS is associated with reduced expression of COMT gene products in peripheral cells, with individuals having approximately 50% less COMT mRNA, COMT protein expression and enzyme activity compared to normal comparison subjects; (ii) the Val158Met genotype had predicted but weak effects on COMT biology in 22q11.2DS, as previously reported in non-hemizygous individuals (14); (iii) identification of haplotypes consisting of previously reported risk and functional polymorphisms were critical for modulating the Val158Met effect on COMT expression and enzyme activity, and (iv) rs165599, a 3UTR SNP previously linked with schizophrenia and relatively reduced expression of COMT mRNA, was associated with the presence of psychosis and reduced PIQ in our 22q11.2DS sample, with the G risk-associated allele again being associated with low levels of COMT expression, possibly because of a specific effect on an extended 3′UTR *COMT* transcript.

Our data demonstrate that COMT protein and enzyme activity is predictably reduced by 50% in 22q11.2DS, consistent with previous findings showing that COMT mRNA is under-expressed by ~50% in mice models and humans with 22q11.2DS (38, 39) and extending these findings to COMT enzyme activity. The fact that 22q11.2DS and age- and sex-matched controls are derived from different ethnic populations (Jewish-Israeli and European ancestry, respectively) and the small sample size of the controls are potential limitations. Yet, as hypothesized, we found ~50% reduction in 22q11.2DS compared to controls in COMT mRNA levels (47% reduction), S-COMT protein levels (44% reduction), and enzyme activity (43% reduction), suggesting that our findings accurately reflect haploinsufficiency of the *COMT* gene in 22q11.2DS.

We found an age effect on S-COMT and MB-COMT levels and enzyme activity. The age effect was in line with previous reports of negative correlations between age and COMT biological activity (40). Interestingly, our work was conducted with lymphoblasts which are

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

all of the same age with respect to their passages as cell lines. It suggests that the cell lines maintain an 'epigenetic memory', i.e., age associated epigenetic marks that retained the age of the subjects that the cells originally came from and that are not lost in the transformation and in the passages of the cell lines (41).

Our analysis of a 3 marker haplotype consisting of rs2075507, rs4680 and rs165599, which had previously been associated with risk for schizophrenia in non-22q11.2DS individuals (11), revealed a striking phenotypic separation of Val-containing haplotypes involved in COMT biology and enzyme activity. Hemizygous Val-containing (AGA) chromosomes were associated with the highest levels of COMT mRNA, S-COMT and MB-COMT proteins and of enzyme activity compared to all other groups, including Val-containing AGG chromosomes. The AGG Val-containing chromosome was indistinguishable from all Met-containing haplotypes at the biological level. This is consistent with the predicted biological summation of the effects of the high activity Val allele with the low expression G allele at rs165599 and suggest that rs165599 or variants in linkage disequilibrium with it (e.g. rs3838146, see below) modulate effects of the Val allele and differentiates two populations of Val-containing chromosomes. Interestingly, genetic variation at rs165599 had no modifying effect on Met-containing chromosomes. The observation that the rs165599 G allele-containing version of these haplotypes has the lowest levels of COMT expression and activity in 22q11.2DS is consistent with previous studies of rs165599 in non-hemizygous individuals, which showed that the G-G genotype (Val and rs165599) was associated with the lowest levels of mRNA expression in normal human cortical tissue (22).

The rs165599 resides in an extended 3′UTR and is present in a small number of full-length COMT human mRNA sequences in Genbank (AK130031 and AK290440), as well as being transcribed and present in COMT transcripts with an extended 3′UTR (22). This is consistent with our findings that the rs165599 genotype significantly and perhaps specifically influences COMT-extended mRNA expression in 22q11.DS and supports a functional impact of the SNP on mRNA expression. In addition, our results confirm the association of rs165599 with psychosis in 22q11.DS, consistent with previous reports in non-22q11.2DS schizophrenia (11), and further suggest that this SNP is associated with cognitive differences in 22q11.2DS, with individuals who have lower levels of COMT mRNA expression (G) also having significantly lower IQ levels and higher risk for psychosis.

We observed an uncommon single nucleotide – 'T' variant of *COMT* rs74745580 in two of the 53 22q11.2DS individuals (3.8%). The rs74745580 has two alleles, 'C' (with a frequency of 0.997–0.999 and 'T' (with a frequency of 0.001–0.003 in European samples) (http://www.1000genomes.org and http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=74745580). This variant appears to be more frequent in our Jewish sample. Here, we show for the first time that the 'T' mutation is associated with the absence of S-COMT expression and very low COMT activity in 22q11.2DS (Figure 5). Both of the individuals with the mutation had comorbid neuropsychiatric diagnoses but not schizophrenia, one with autism and non-specific psychosis, and the other with ADHD and depression, demonstrating no clear association to a specific psychiatric phenotype in this small sample.

Analyses of the Val158Met polymorphism on COMT protein expression and enzyme activity revealed relatively small differences in S-COMT levels and enzyme activity (14% and 8%, respectively) in 22q11.2DS. We also report a significant association of Val158Met with COMT-common mRNA expression levels, whereby the Val allele was associated with higher levels of expression, consistent with that reported by Bray *et al*. (22) in human brain. The magnitude of the Val158Met effect on enzyme activity was smaller than in previous findings in healthy subjects which showed that Val/Val carriers have a ~40% higher S-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

COMT enzyme activity than Met/Met carriers (14), possibly due to the interaction of functional coding variants and also of cis-regulatory SNPs that influence COMT activity. Specifically, alleles at rs4860 and rs4818 were in perfect identity so that the 'A' (Met) low activity allele always segregated with the 'C' high activity allele of rs4818 (14, 25). The rs4860 locus influences COMT protein stability while the rs4818 locus affects COMT translation efficiency (14, 25). Thus, the Met allele could potentially be compensated by the rs4818 'C' allele in our cases, which may explain the relatively small effect of Val158Met alone in our sample.

Likewise, the effect of the rs165599 3′UTR SNP could not be differentiated from the effect of the rs3838146 indel variation near it in the 3′UTR, as they, too, were identical in their allele distributions. Whereas rs165599 presumably affects COMT by altering microRNA-mediated RNA processing, the potential mechanism of rs3838146 indel on COMT is not known.

We also examined the effect of an 8-marker TXNRD2-COMT-ACVRF haplotype on COMT function. This haplotype contains the 3-marker haplotype backbone plus 5 additional putative functional polymorphisms associated with COMT/ACVRF expression or schizophrenia (24). Consistent with the 3-marker haplotype association, we observed that two separate Val-containing chromosomes could be defined in 22q11.2DS, (HAP1 and HAP2) with different COMT protein levels and marginally different enzyme activity levels. Yet, it is important to note that the 8-marker haplotype analyses are statistically underpowered in this sample.

In the current 22q11.2DS sample, we failed to find any association between Val158Met with psychosis. Of note, an earlier *COMT* Met association with psychosis in 22q11.2DS was reported on a different smaller sample (6). No association between *COMT* Val158Met and schizophrenia was found in two additional 22q11.2DS cohorts (7, 34). The results of our study suggest that the inconsistencies in *COMT* Val158Met findings may be the result of the substantial genetic and biological heterogeneity within Val-containing chromosomes, confounding simple single locus Val158Met effects.

Although MB-COMT is expressed at higher levels than S-COMT in the brain, we found more significant effects of the S-COMT than the MB-COMT isoform in our peripheral cell assays. We believe this is related to limitations of laboratory methods on peripheral cells, particularly related to accurate measurement of MB COMT, the primary brain form of COMT. The enzyme assay tends to reflect primarily S-COMT because the MB form is less soluble in the lysis buffer. In addition, the MB-COMT antibody has much less sensitivity than the S-COMT antibody.

Overall, our results support the conclusion that *COMT* variants other than Val158Met are of functional importance in determining COMT biology and possibly also neuropsychiatric risk and that Val containing chromosomes defined by Val158Met genotypes are biologically heterogeneous. While we have not differentiated the effects of all possible combinations of functional alleles in *COMT*, we did find that the 3-marker haplotype (AGA) is associated with high activity *COMT*-related phenotypes and that the rs165599 'G' allele is associated with low COMT expression and increased risk for psychosis. The only difference between the 'high Val' (AGA haplotype) and the 'low Val' (AGG haplotype) groups stem from the A/G polymorphism of rs165599 suggesting that rs165599 is directly effecting COMT biology and the risk for psychosis and cognitive deficits. Yet, it could also be that a variant in linkage disequilibrium with rs165599 (e.g. rs3838146) differentiate the two *COMT* Val subpopulations. It is also important to note that the influence of rs165599 appears to depend on the haplotypic background, as genetic variation at rs165599 had no modifying effect on

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Met-containing chromosomes. It is worth noting that the GAA three marker haplotype, i.e. the A allele at rs165599 on the Met background, also had relatively low mRNA expression and enzyme activity, yet this haplotype was not associated with increased risk of psychosis or cognitive deficits. This suggests that low COMT activity per se, at least at the whole peripheral cell level and as assayed herein, is not a reliable predictor of psychosis risk. It is conceivable that the apparently selective effect of rs165599 on the extended 3′ UTR COMT transcript in the context of specific coding alleles on that transcript is a clue to another level of complexity of the psychosis related aspects of COMT. This transcript is presumably trafficked differently than the shorter 3′UTR versions, perhaps being associated with cell machinery more relevant to psychosis than the shorter 3′UTR transcript.

Our study was relatively large in comparison to prior biological studies of 22q11.2DS yet limited when divided into the different haplotype subgroups. Therefore, it will be important to expand the current sample to replicate our findings and to further disambiguate the biological interactions between loci in this gene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the March of Dimes (grant no. 5-FY06-590 to DG) and direct funding of the Weinberger Lab at the NIMH Intramural Research Program.

References

1. Shprintzen RJ. Velo- cardio-facial syndrome: A distinctive behavioral phenotype. Mental retardation and developmental disabilities research reviews. 2000; 6:142–147. [PubMed: 10899808]
2. Green T, Gothelf D, Glaser B, Debbane M, Frisch A, Kotler M, et al. Psychiatric Disorders and Intellectual Functioning Throughout Development in Velocardiofacial (22q11.2 Deletion) Syndrome. J Am Acad Child Adolesc Psychiatry. 2009; 48:1060–1068. [PubMed: 19797984]
3. Ho JS, Radoeva PD, Jalbrzikowski M, Chow C, Hopkins J, Tran WC, et al. Deficits in Mental State Attributions in Individuals with 22q1 1. 2 Deletion Syndrome (Velo-Cardio-Facial Syndrome). Autism Res. 2012
4. Antshel KM, Shprintzen R, Fremont W, Higgins AM, Faraone SV, Kates WR. Cognitive and psychiatric predictors to psychosis in velocardiofacial syndrome: a 3-year follow-up study. J Am Acad Child Adolesc Psychiatry. 2010; 49:333–344. [PubMed: 20410726]
5. Swillen A, Devriendt K, Legius E, Eyskens B, Dumoulin M, Gewillig M, et al. Intelligence and psychosocial adjustment in velocardiofacial syndrome: a study of 37 children and adolescents with VCFS. J Med Genet. 1997; 34:453–458. [PubMed: 9192263]
6. Gothelf D, Eliez S, Thompson T, Hinard C, Penniman L, Feinstein C, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. Nat Neurosci. 2005; 8:1500–1502. [PubMed: 16234808]
7. Murphy KC, Jones LA, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. Arch Gen Psychiatry. 1999; 56:940–945. [PubMed: 10530637]
8. Rodriguez-Murillo L, Gogos JA, Karayiorgou M. The genetic architecture of schizophrenia: new mutations and emerging paradigms. Annu Rev Med. 2012; 63:63–80. [PubMed: 22034867]
9. Karayiorgou M, Simon TJ, Gogos JA. 22q11. 2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. Nat Rev Neurosci. 2010; 11:402–416. [PubMed: 20485365]
10. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA. 2001; 98:6917–6922. [PubMed: 11381111]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

11. Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, et al. A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet. 2002; 71:1296–1302. [PubMed: 12402217]
12. Strous RD, Nolan KA, Lapidus R, Diaz L, Saito T, Lachman HM. Aggressive behavior in schizophrenia is associated with the low enzyme activity COMT polymorphism: a replication study. Am J Med Genet B Neuropsychiatr Genet. 2003; 120B:29–34. [PubMed: 12815735]
13. Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry. 2006; 60:141–151. [PubMed: 16476412]
14. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet. 2004; 75:807–821. [PubMed: 15457404]
15. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics. 1996; 6:243. [PubMed: 8807664]
16. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry. 1995; 34:4202–4210. [PubMed: 7703232]
17. Drabant EM, Hariri AR, Meyer-Lindenberg A, Munoz KE, Mattay VS, Kolachana BS, et al. Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. Arch Gen Psychiatry. 2006; 63:1396. [PubMed: 17146014]
18. Heinz A, Smolka MN. The effects of catechol O-methyltransferase genotype on brain activation elicited by affective stimuli and cognitive tasks. Rev Neurosci. 2006; 17:359. [PubMed: 16878403]
19. Bertolino A, Rubino V, Sambataro F, Blasi G, Latorre V, Fazio L, et al. Prefrontal-hippocampal coupling during memory processing is modulated by COMT val158met genotype. Biol Psychiatry. 2006; 60:1250–1258. [PubMed: 16950222]
20. Käenmäki M, Tammimäki A, Myöhänen T, Pakarinen K, Amberg C, Karayiorgou M, et al. Quantitative role of COMT in dopamine clearance in the prefrontal cortex of freely moving mice. J Neurochem. 2010; 114:1745–1755. [PubMed: 20626558]
21. Allen NC, Bagade S, McQueen MB, Ioannidis JPA, Kavvoura FK, Khoury MJ, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet. 2008; 40:827–834. [PubMed: 18583979]
22. Bray NJ, Buckland PR, Williams NM, Williams HJ, Norton N, Owen MJ, et al. A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. Am J Hum Genet. 2003; 73:152–161. [PubMed: 12802784]
23. Gong Y, Wu CN, Xu J, Feng G, Xing Q, Fu W, et al. Polymorphisms in microRNA target sites influence susceptibility to schizophrenia by altering the binding of miRNAs to their targets. European Neuropsychopharmacology. 2013
24. Michaelovsky E, Gothelf D, Korostishevsky M, Frisch A, Burg M, Carmel M, et al. Association between a common haplotype in the COMT gene region and psychiatric disorders in individuals with 22q11.2DS. Int J Neuropsychopharmacol. 2008; 11:351–363. [PubMed: 17949513]
25. Nackley A, Shabalina S, Tchivileva I, Satterfield K, Korchynskyi O, Makarov S, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science. 2006; 314:1930–1933. [PubMed: 17185601]
26. Murphy BC, O'Reilly RL, Singh SM. Site-specific cytosine methylation in S-COMT promoter in 31 brain regions with implications for studies involving schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2005; 133:37–42. [PubMed: 15635661]
27. Ursini G, Bollati V, Fazio L, Porcelli A, Iacovelli L, Catalani A, et al. Stress-related methylation of the catechol-O-methyltransferase Val158 allele predicts human prefrontal cognition and activity. J Neurosci. 2011; 31:6692–6698. [PubMed: 21543598]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

28. Glaser B, Debbane M, Hinard C, Morris MA, Dahoun SP, Antonarakis SE, et al. No evidence for an effect of COMT Val158Met genotype on executive function in patients with 22q11 deletion syndrome. Am J Psychiatry. 2006; 163:537–539. [PubMed: 16513880]
29. Gothelf D, Hoeft F, Hinard C, Hallmayer JF, Stoecker JV, Antonarakis SE, et al. Abnormal cortical activation during response inhibition in 22q11.2 deletion syndrome. Hum Brain Mapp. 2007; 28:533–542. [PubMed: 17427209]
30. Gothelf D, Michaelovsky E, Frisch A, Zohar AH, Presburger G, Burg M, et al. Association of the low-activity COMT 158Met allele with ADHD and OCD in subjects with velocardiofacial syndrome. Int J Neuropsychopharmacol. 2007; 10:301–308. [PubMed: 16734939]
31. Takarae Y, Schmidt L, Tassone F, Simon TJ. Catechol-O-methyltransferase polymorphism modulates cognitive control in children with chromosome 22q11.2 deletion syndrome. Cogn Affect Behav Neurosci. 2009; 9:83–90. [PubMed: 19246329]
32. Baker K, Baldeweg T, Sivagnanasundaram S, Scambler P, Skuse D. COMT Val108/158 Met modifies mismatch negativity and cognitive function in 22q11 deletion syndrome. Biol Psychiatry. 2005; 58:23–31. [PubMed: 15935994]
33. Gothelf D, Eliez S, Thompson T, Hinard C, Penniman L, Feinstein C, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. Nat Neurosci. 2005; 8:1500–1502. [PubMed: 16234808]
34. Bassett AS, Caluseriu O, Weksberg R, Young DA, Chow EW. Catechol-O-methyl transferase and expression of schizophrenia in 73 adults with 22q11 deletion syndrome. Biol Psychiatry. 2007; 61:1135–1140. [PubMed: 17217925]
35. Li T, Ball D, Zhao J, Murray R, Liu X, Sham P, et al. Family-based linkage disequilibrium mapping using SNP marker haplotypes: application to a potential locus for schizophrenia at chromosome 22q11. Mol Psychiatry. 2000; 5:77. [PubMed: 10673772]
36. Meyer-Lindenberg A, Nichols T, Callicott JH, Ding J, Kolachana B, Buckholtz J, et al. Impact of complex genetic variation in COMT on human brain function. Mol Psychiatry. 2006; 11:867–877. [PubMed: 16786032]
37. Zürcher G, Prada M. Rapid and Sensitive Single-Step Radiochemical Assay for Catechol-O-Methyltransferase. J Neurochem. 1982; 38:191–195. [PubMed: 7108526]
38. Meechan DW, Tucker ES, Maynard TM, LaMantia AS. Diminished dosage of 22q11 genes disrupts neurogenesis and cortical development in a mouse model of 22q1 1 deletion/DiGeorge syndrome. Proc Natl Acad Sci. 2009; 106:16434–16445. [PubMed: 19805316]
39. Sivagnanasundaram S, Fletcher D, Hubank M, Illingworth E, Skuse D, Scambler P. Differential gene expression in the hippocampus of the *Df1*/+ mice: A model for 22q11. 2 deletion syndrome and schizophrenia. Brain Res. 2007; 1139:48–59. [PubMed: 17292336]
40. Tunbridge EM, Weickert CS, Kleinman JE, Herman MM, Chen J, Kolachana BS, et al. Catechol-o-methyltransferase enzyme activity and protein expression in human prefrontal cortex across the postnatal lifespan. Cereb Cortex. 2007; 17:1206–1212. [PubMed: 16835293]
41. Akbarian S, Beeri MS, Haroutunian V. Epigenetic Determinants of Healthy and Diseased Brain Aging and CognitionDeterminants of Healthy and Diseased Brain Aging. JAMA neurology. 2013; 70:711–718. [PubMed: 23571692]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

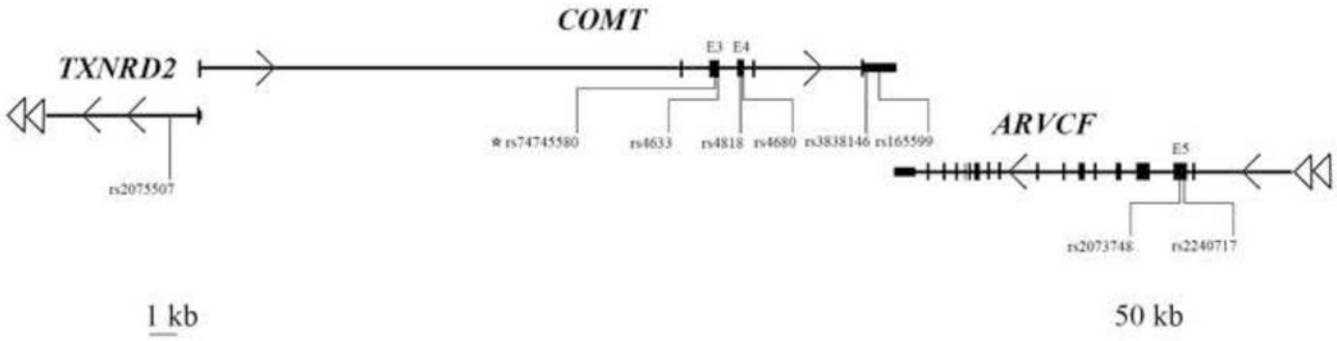

Figure 1.
Diagram of the *TXNRD2*, *COMT* and *ARVCF* genes showing locations of genotyped single nucleotide polymorphisms.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

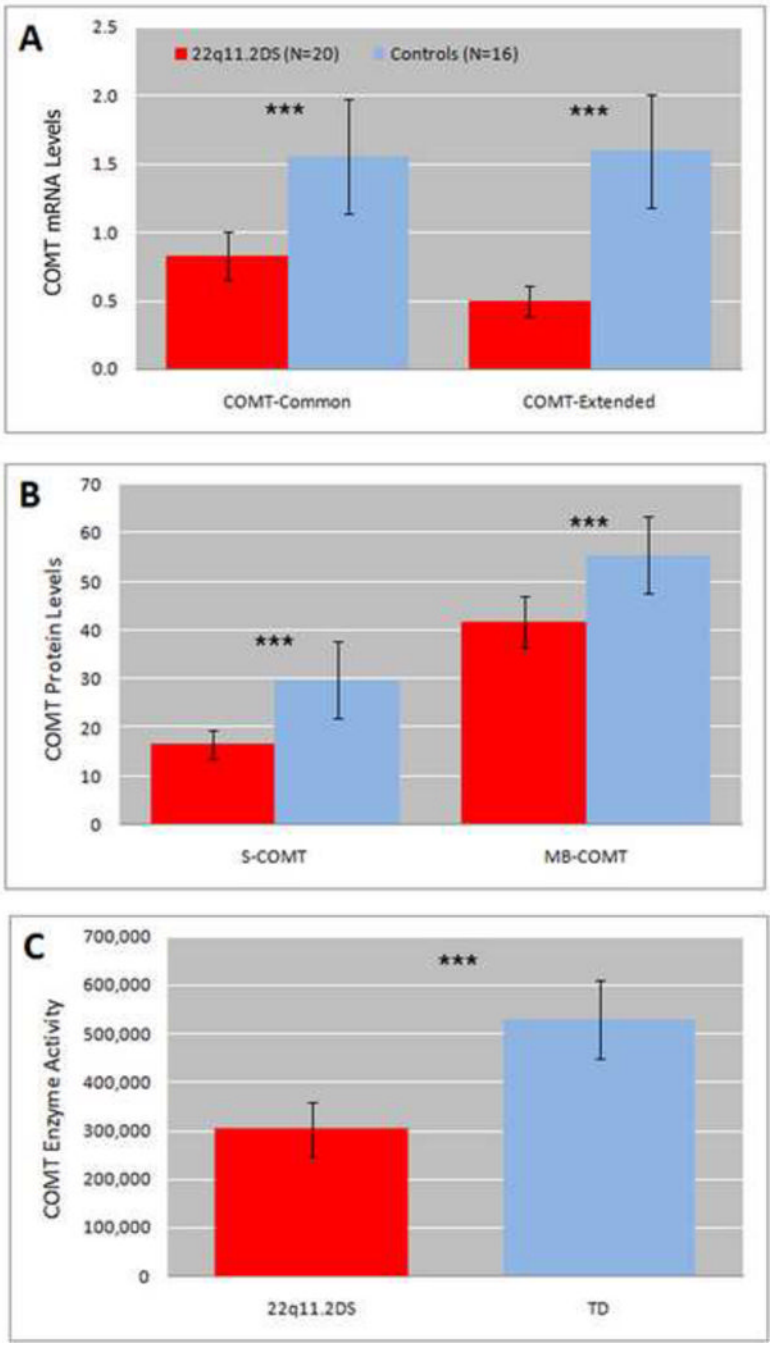

Figure 2.

Comparison between 22q11.2DS and typically developing controls with normalized levels. **(A)** There was a significant effect of diagnosis on mRNA expression levels of all COMT transcripts (COMT-common, $F(1, 33) = 133$; $p < .0001$) and on COMT transcripts containing an extended 3-UTR (COMT-extended, $F(1, 33) = 47.2$; $p < .0001$). As expected, 22q11.2DS individuals exhibited lower COMT-extended and COMT-common mRNAs levels (69% and −47%, respectively). **(B)** The immunoblotting analysis revealed that COMT proteins migrated as a double band at the predicted molecular weights of 25 kDa, and 28 kDa, corresponding to soluble COMT (S-COMT) and membrane-bound COMT (MB-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

COMT), respectively. S-COMT and MB-COMT are expressed as optical density units (OD). There was a significant effect of diagnosis on S-COMT ($F(1, 32) = 48.7$; $p < .0001$) and MB-COMT levels ($F(1, 33) = 41.8$; $p < .0001$), with 22q11.2DS patients having 44% and 25% lower levels of S-COMT and MB-COMT, respectively, compared to controls. **(C)** Relative COMT enzyme activity is presented as disintegrations per minute (dpm) per mg total protein. As expected, COMT enzyme activity was also significantly (43%) lower in 22q11.2DS patients compared to controls [($F(1, 34) = 120$; $p < .0001$]. ***$p < .0001$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

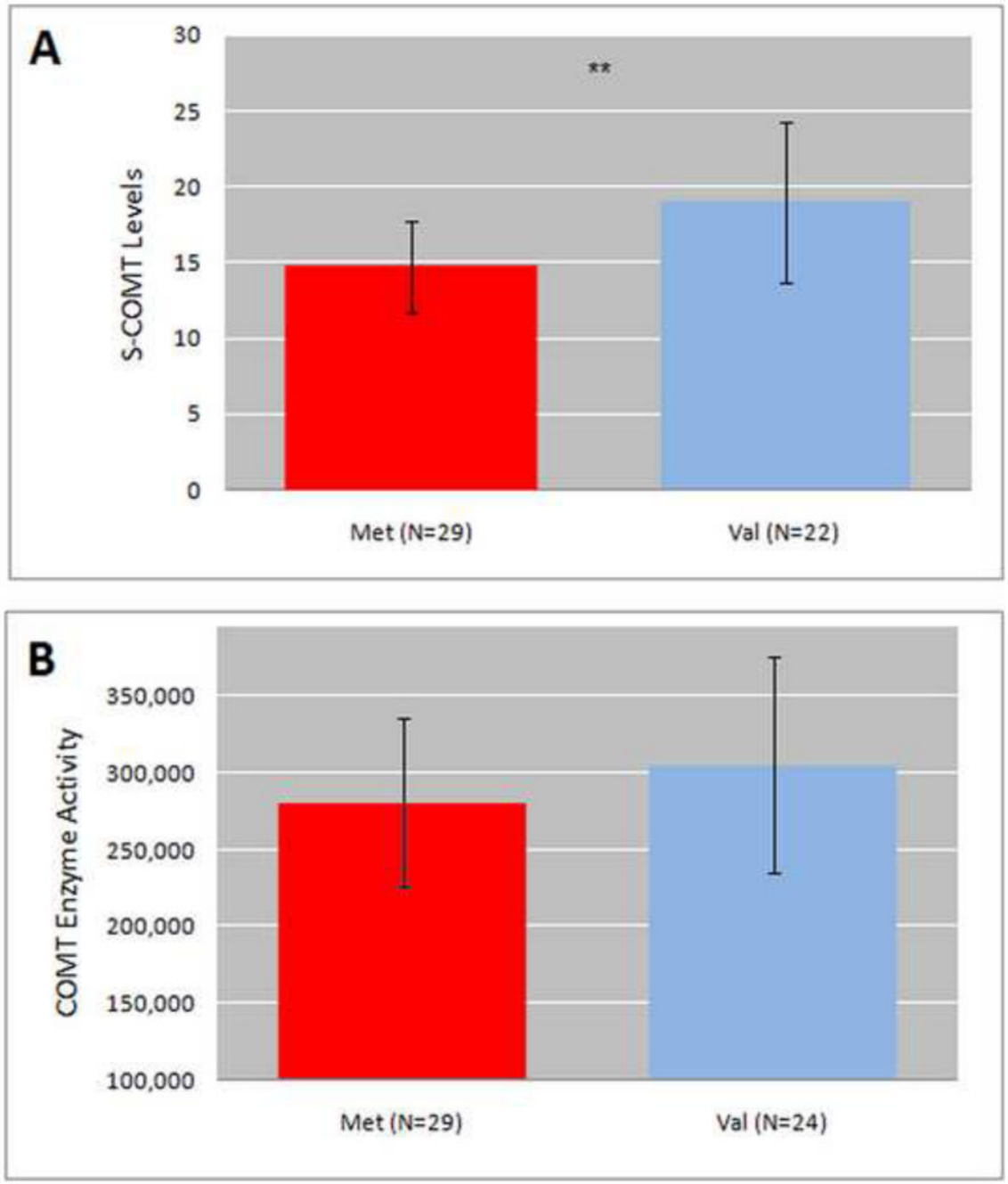

Figure 3.

Comparison of soluble COMT (S-COMT) protein levels and COMT enzyme activity between 22q11.2DS *COMT* Met vs. Val carriers (A) A significant COMT genotype effect was observed for S-COMT protein levels ($F(1, 49)=13.1$; $p=0.001$), with COMT Met individuals exhibiting 23% lower levels compared to Val carriers. (B) There was no significant difference in COMT enzyme activity in COMT Met vs. Val carriers ($F(1, 51)=2.0$; $p=0.16$), although slightly lower levels (10%) were observed in *COMT* Met allele carriers.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

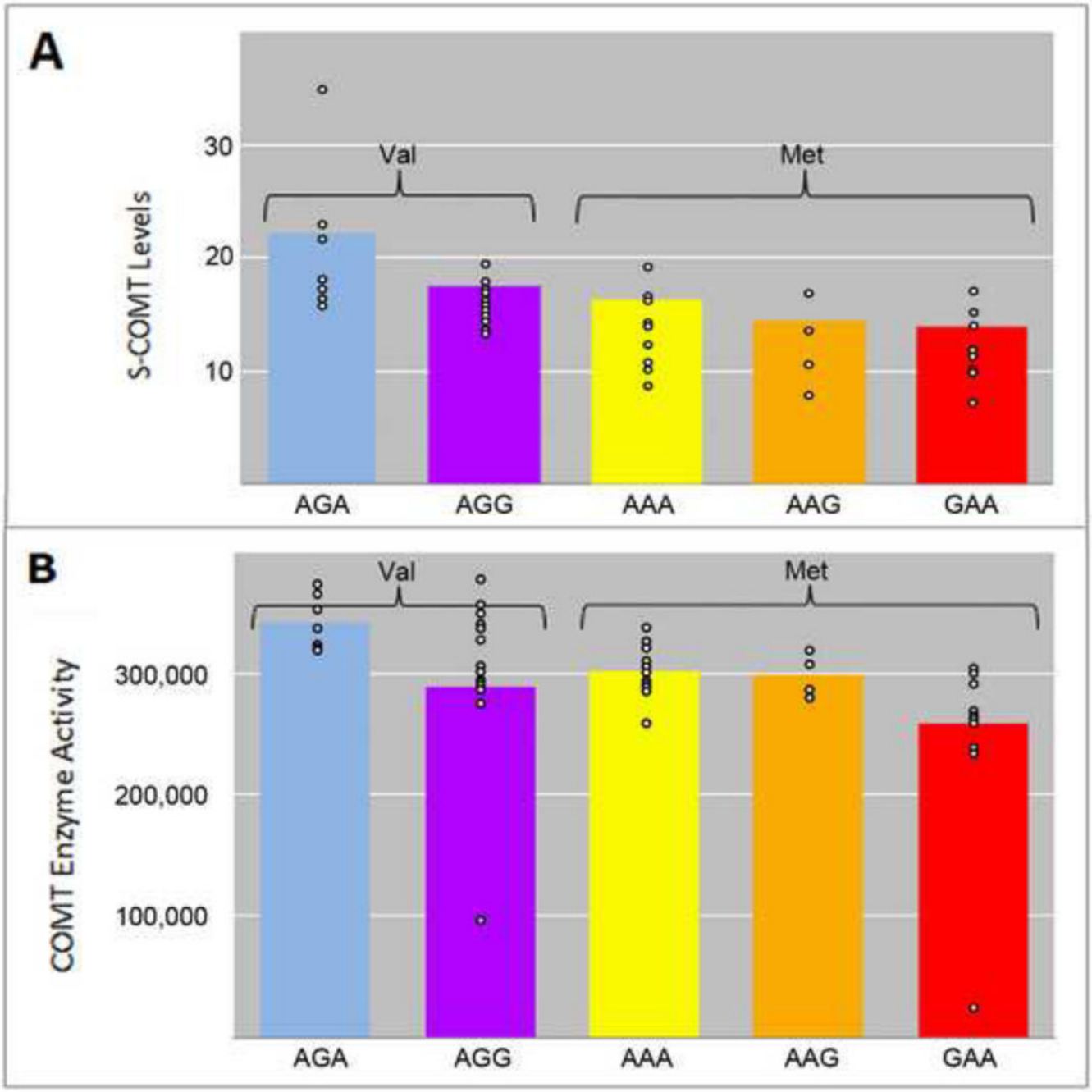

Figure 4.

Effects of the 3 Markers Haplotype (rs2075507-rs4680-rs165599) on S-COMT levels (Panel **A**) and COMT enzyme activity (Panel **B**). A highly significant effect of the 3-marker haplotypes was observed on S-COMT levels ($F(4,46) = 6.2$; $p < .0001$). Post hoc analysis revealed that the COMT AGA haplotype had significantly higher S-COMT levels compared to the other 4 haplotypes (Panel **A**). COMT enzyme activity also significantly differed among the 3 COMT marker haplotypes ($F(4,48) = 2.4$; $p < .05$), with the COMT AGA haplotype showing significantly higher enzyme activity levels compared to the AGG and GAA haplotypes (Panel **B**).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

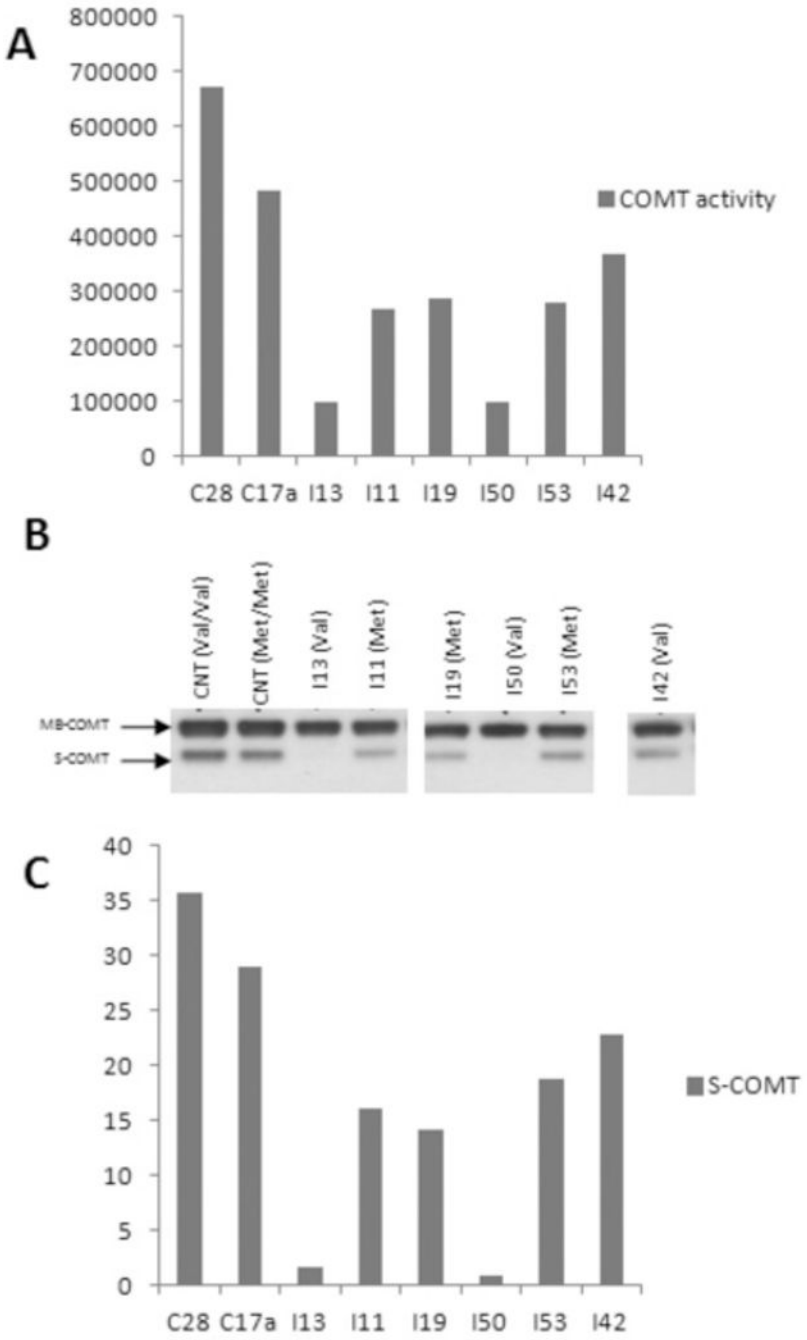

Figure 5.

(A) Low COMT activity and **(B)** and **(C)** loss of S-COMT protein in two 22q11.2DS subjects with the 'T' allele of rs74745580 (I13, I-50) compared to two control subjects (C28, C17a) with the 'C-C' genotype and four 22q11.2DS subjects (I-11, 19, 53, 42) with the 'C' allele. COMT rs4680 *Met/Val* genotype is shown for all individuals. S-COMT and MB-COMT protein representative bands are labeled.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

COMT mRNA, Protein Levels and Enzyme Activity in 22q11.2 Deletion Syndrome: Comparison among the 3 Markers Haplotype (*rs2097603-rs4680-rs165599*)

	AGA (N=7)	**AGG (N=17)**	**AAA (N=11)**	**AAG (N=4)**	**GAA (N=14)**	**Statistics**
Age	19.3 ± 12.7	21.7 ± 9.3	19.2 ± 7.1	15.8 ± 9.7	18.1 ± 8.1	F = 0.5, *p* = .73
Males / Females	4 / 3	9 / 8	6 / 5	3 / 1	9 / 5	*p* = .915
COMT-EXTENEDED	0.6 ± 0.3	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	F (df = 4, 48) = 2.8, *p* = .03 AAA** & AGA*> AGG
COMT-COMMON	0.92 ± 0.9	0.82 ± 0.1	0.79 ± 0.2	0.78 ± 0.3	0.65 ± 0.3	F (df = 4, 48) = 2.4, *p* = .07 AGG* & AGA*> GAA
S-COMT	22.3 ± 8.6	17.4 ± 1.6	16.2 ± 2.9	14.4 ± 3.5	13.7 ± 2.7	F (df = 4, 46)= 6.2, *p* < .0001 AGA> AAA**, AAG**, AGG**, GAA***; AGG>GAA*
MB-COMT	46.8 ± 7.2	41.0 ± 4.8	44.8 ± 7.1	38.2 ± 5.4	41.1 ± 4.2	F (df = 4, 48) = 2.6, *p* = .05 AGA>AAG*, AGG*, GAA*; AAA>AAG*
Enzyme activity	342852 ± 21502	289759 ± 77527	301989 ± 21735	299282 ± 18017	258801 ± 70713	F (df = 4, 48) = 2.4, *p* = .05 AGA> AGG*, GAA**

22q1 1.2DS=22q11.2 deletion syndrome; COMT-COMMON=all transcripts of COMT mRNA; COMT-EXTENDED=extended COMT long 3′UTR mRNA; MB-COMT=membrane bound COMT protein; S-COMT,=soluble COMT protein.

* p < .05,

** p < .01,

*** p < .001