Biological Psychiatry: Celebrating 50 Years

Archival Report

Which Dopamine Polymorphisms Are Functional? Systematic Review and Meta-analysis of *COMT*, *DAT*, *DBH*, *DDC*, *DRD1–5*, *MAOA*, *MAOB*, *TH*, *VMAT1*, and *VMAT2*

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ABSTRACT

BACKGROUND: Many polymorphisms in dopamine genes are reported to affect cognitive, imaging, or clinical phenotypes. It is often inferred or assumed that such associations are causal, mediated by a direct effect of the polymorphism on the gene product itself. However, the supporting evidence is not always clear.

METHODS: We conducted systematic reviews and meta-analyses to assess the empirical evidence for functional polymorphisms in genes encoding dopaminergic enzymes (*COMT*, *DBH*, *DDC*, *MAOA*, *MAOB*, and *TH*), dopamine receptors (*DRD1*, *DRD2*, *DRD3*, *DRD4*, and *DRD5*), the dopamine transporter (*DAT*), and vesicular transporters (*VMAT1* and *VMAT2*). We defined functionality as an effect of the polymorphism on the expression, abundance, activity, or affinity of the gene product.

RESULTS: We screened 22,728 articles and identified 255 eligible studies. We found robust and medium to large effects for polymorphisms in 4 genes. For catechol-*O*-methyltransferase (*COMT*), the Val^{158}Met polymorphism (rs4680) markedly affected enzyme activity, protein abundance, and protein stability. Dopamine β-hydroxylase (DBH) activity was associated with rs1611115, rs2519152, and the *DBH*–STR polymorphism. Monoamine oxidase A (MAOA) activity was associated with a 5′ VNTR polymorphism. Dopamine D_2 receptor (DRD2) binding was influenced by the Taq1A (rs1800497) polymorphism, and rs1076560 affected *DRD2* splicing.

CONCLUSIONS: Some widely studied dopaminergic polymorphisms clearly and substantially affect the abundance or activity of the encoded gene product. However, for other polymorphisms, evidence of such an association is negative, inconclusive, or lacking. These findings are relevant when selecting polymorphisms as “markers” of dopamine function, and for interpreting the biological plausibility of associations between these polymorphisms and aspects of brain function or dysfunction.

Keywords: Catechol-*O*-methyltransferase, Dopamine receptor, Dopamine transporter, Gene expression, Monoamine oxidase, Variant

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Dopamine regulates diverse aspects of brain function (1–4) and plays roles in multiple psychiatric and neurological conditions (5–7). In an attempt to interrogate links between dopamine and human phenotypes, many studies report statistical associations between single nucleotide polymorphisms (SNPs) or other polymorphic variants in dopamine genes and a phenotype of interest, such as performance on a cognitive test [e.g., Farrell *et al.* (8)], a neuropsychiatric diagnosis [e.g., Franke *et al.* (9)], or a neuroimaging measure [e.g., Meyer-Lindenberg *et al.* (10)]. Such associations are, a priori, more plausible if the polymorphism in question is known to be "directly" functional; i.e., it measurably impacts on a biological parameter of the gene’s function. However, in many instances this is not the case: the polymorphism is noncoding (e.g., it is intronic, or within an untranslated region) or it lacks independent evidence of any physiological or pathological correlates. Rightly, this contributes to concerns that many dopaminergic genetic associations with "downstream" phenotypes (such as disease risk, cognitive performance, or blood oxygen level–dependent signal) may be false-positives. Even in the case of polymorphisms that are cited as being functional, the underlying data are sometimes sparse, inconsistent, or limited to in vitro models or in silico predictions. Here, we systematically investigate the evidence for direct functional effects of polymorphisms in all the key human dopamine genes (i.e., those encoding synthetic and catabolic enzymes, receptors, and vesicular and reuptake transporters).

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METHODS AND MATERIALS

We registered the study protocol on the PROSPERO international register of systematic reviews (http://www.crd.york.ac.uk/prospero/DisplayPDF.php?ID=CRD42015029908), where full information about the methods of the systematic review and meta-analysis are reported. We selected 14 genes integrally involved in dopamine transmission, i.e., those that code for its 5 receptors (*DRD1–DRD5*); the dopamine transporter (*DAT*); the vesicular monoamine transporters (*VMAT1* and *VMAT*); and the enzymes that synthesize it (tyrosine hydroxylase [*TH*] and DOPA decarboxylase [*DDC*]) and metabolize it (catechol-*O*-methyltransferase [*COMT*], dopamine β-hydroxylase [*DBH*], and monoamine oxidase A and B [*MAOA* and *MAOB*]). We acknowledge that this is necessarily a somewhat arbitrary list, since some of these genes also affect nondopamine parameters (e.g., *VMAT*s and *MAOB* affect 5-hydroxytryptamine) and, conversely, dopamine function may be affected by some genes that we did not include.

Our objective was to identify evidence of associations between sequence variation (SNPs, restriction fragment length polymorphisms, repeat polymorphisms, or insertion/deletion polymorphisms) in or near each gene, and the functionality of its gene product. We defined "functionality" as comprising the following: empirical effects of the genetic variant on the gene's expression (e.g., methylation status, promoter activity, transcription factor binding); the abundance of the encoded messenger RNA (mRNA) isoform(s); measures of the abundance of the encoded protein (e.g., immunoreactivity, radioligand binding, stability); and the function or activity of the encoded protein (e.g., enzyme activity). We did not consider studies that predicted functional effects in silico. Nor did we consider studies that examined indirect measures of gene product function or pharmacogenetic effects, nor those reporting an interaction between genotype and another factor (e.g., medication, stress) on function. We considered all polymorphisms with a global minor allele frequency of ≥1% as reported in dbSNP (https://www.ncbi.nlm.nih.gov/snp), and thus we did not investigate rare variants. It was beyond our scope to include functional effects of a haplotype rather than individual SNPs or unpublished findings from expression quantitative trait locus datasets.

Search Strategy

We searched Web of Science (All Databases, refined to Web of Science Core Collection and MEDLINE) for Field: Topic (i.e., title, abstract, keywords) using the search terms "(Gene name(s) OR gene symbol(s)) AND (SNP OR polymorphi* OR allel* or genotyp* OR variant* OR VNTR OR RFLP OR duplication*)" from inception until August 2, 2018. The names and symbols used for each gene were as follows: for dopamine receptors: ("D*n* receptor" OR "D*n* dopamine receptor" OR "D*n*R" OR "DRD*n*"), where *n* = 1 to 5; for *COMT*: ("COMT" OR "catechol-o-methyl*"); for *DAT*: ("Dopamine transporter" OR "DAT" OR "SCL6A3"); for *TH*: ("Tyrosine *hydroxylase" OR TH); for *DDC*: ("DDC" OR "AADC" OR "AAAD" OR "DOPA decarboxylase" OR "aromatic L-amino acid decarboxylase"); for *DBH*: ("DBH" OR "dopamine *-hydroxylase"); for *MAO*: ("MAO*n*" OR "MAO-*n*" OR "monoamine oxidase *n*"), where *n* = 1 or 2; for *VMAT1*: ("VMAT1" OR "vesicular monoamine transporter 1" OR "SLC18A1" OR "CGAT"); for *VMAT2*: ("VMAT2" OR "vesicular monoamine transporter 2" OR "SLC18A2"). Each search and data extraction was conducted independently by EMT or PJH, and at least 1 other author. In addition, we searched our own reprint collections and the reference lists of identified studies. Any discrepancies were resolved by EMT and PJH.

Meta-analysis

We conducted pairwise meta-analyses using Review Manager (version 5.3), the software used for preparing and maintaining Cochrane Reviews (https://community.cochrane.org/help/tools-and-software/revman-5), and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (11). In accordance with PROSPERO protocol, we performed meta-analyses where ≥3 studies had measured the effect of a given polymorphism on the same or similar parameter (12). For example, we meta-analyzed positron emission tomography and single photon emission computerized tomography measures of binding potential together with postmortem measurement of binding site density. In studies that examined a genotype effect on a parameter in multiple brain regions, we selected the region with the largest sample size, or we selected the largest region (e.g., whole striatum rather than caudate or putamen), or we combined regions for an average value according to the formula in the Cochrane Handbook (13). We did not meta-analyze studies in which variance measures were obtained from technical replicates (e.g., luciferase assays) or in which data were categorical (as was the case for most methylation data). Most standard systematic review indices of risk of bias are not applicable to the types of study included here (e.g., randomization, clinical exclusion criteria) or else were not recorded (e.g., whether experiments were conducted blind to genotype).

We extracted graphical data using WebPlotDigitizer (14). Only data presented as means with a measure of variance could be meta-analyzed; where necessary, we converted standard errors of the mean (or confidence intervals) to standard deviations according to guidance in the Cochrane Handbook (13). Where data were not presented in this way or if the findings were ambiguous (e.g., genotype distributions, overlap with findings from another article), we contacted authors to request clarification or the original data. Where studies reported case (e.g., schizophrenia) and healthy control groups separately, we included only the latter data, but we included case data if cases and controls were grouped together. We did not meta-analyze datasets composed entirely of clinical cases (i.e., those that did not include any healthy control individuals), since genotype effects could be confounded by gene-by-diagnosis interactions and by medication and other confounders. We combined minor allele carriers with heterozygotes and compared these against major allele homozygotes. In the case of *MAOA* and *MAOB*, located on the X chromosome, only homozygous female subjects were meta-analyzed, to allow them to be pooled with male hemizygotes. When combining subgroups, we used the formula in the Cochrane Handbook (13). The standardized mean difference, with the corresponding 95% confidence interval, was used as a summary statistic because the included studies measured

outcomes in a variety of ways and with different units. We used the random effects model to incorporate the assumption that the studies could estimate effects that followed a specific distribution across different studies. Where >1 SNP within a gene was found to have a significant functional effect, we determined linkage disequilibrium (R^2) between the SNPs using the LDpair tool in the National Cancer Institute LDlink program (https://ldlink.nci.nih.gov/), including data from all ethnic populations.

In accordance with protocol, funnel plots were performed where ≥10 studies were included in a meta-analysis. Heterogeneity between studies was investigated by the *I*-squared statistic (I^2 ≥50% was considered indicative of heterogeneity), and by visual inspection of the forest plots.

Secondary Analyses

Where primary meta-analyses showed significant genotype effects and sufficient studies reported data for minor allele homozygote and heterozygote groups separately, we examined for an allele dose relationship between the polymorphism and the phenotype of interest. To do this, we used Review Manager (as described in the Meta-analysis subsection) to examine whether both homozygote groups differed from heterozygotes in separate random effect models with standardized mean differences. We also planned to analyze genotype effects on COMT function in male subjects and female subjects separately, given the evidence for gender dimorphisms in COMT and its associations with some phenotypes (15).

To investigate the magnitude of the genotype effect for measures included in the primary meta-analyses, we first converted transformed data to raw values (e.g., squaring the means and standard deviations of square root–transformed data), before normalizing the data so that the mean of the major allele homozygote group was set to 1. We then used random effect models with mean differences to derive estimated mean effect sizes with 95% confidence intervals. Note that this approach could not be used to assess the (nonsignificant) effect of the rs28363170 polymorphism on *DAT* mRNA abundance, as 97% of the weighting loaded onto a single study (because of high variance in the remaining 2 studies that was magnified by the data transformation required).

RESULTS

We carried out systematic reviews and performed meta-analyses of the evidence for functional polymorphisms in 14 human dopamine genes. We defined "functional" as meaning that an empirical study had investigated whether a polymorphism affects the abundance or activity of the encoded gene product (see Methods and Materials).

We identified a total of 22,728 hits (ranging from 73 to 5219 per gene), with 255 eligible studies (ranging from 0 to 54 per gene) included in the systematic reviews. A PRISMA statement for each gene is shown in Figure S1 in Supplement 1. Schematics showing the positions of commonly studied polymorphisms within each gene are shown in Figure S2 in Supplement 1. Table 1 gives an overview of the systematic review findings; the detailed results of each systematic review and listing of the included studies are provided in Tables S1 to S13 in Supplement 2. We retrieved sufficient data (defined as ≥3 studies measuring association between a given polymorphism and a single parameter) to conduct 15 meta-analyses (each based on 3–16 studies, and including 66–1307 subjects). A summary of the meta-analytic findings is shown in Table 2, and an overview of the magnitude of genotype effects is given in Figure 1. Funnel plots are shown in Figure S3 in Supplement 1.

Three Dopamine Degradation Enzyme Genes Contain Functional Polymorphisms

COMT. In *COMT* studies, the most widely studied polymorphism was rs4680 (Val^{158}Met). We found that the G (Val) allele is associated across multiple tissue types with greater protein abundance and stability and with greater enzyme activity, but is not associated with *COMT* mRNA abundance (Figure 2). Although they were not amenable to meta-analysis, we found 3 studies indicating that the A (Met) allele may show greater allelic expression (although this does not appear to affect overall *COMT* mRNA expression) (Table S1 in Supplement 2). In addition, 9 studies examined associations between Val^{158}Met and *COMT* gene methylation, but findings were mixed. Aside from Val^{158}Met, multiple studies examined the potential functional impact of rs165599 and rs737865, but we did not observe any consistent effects across studies (Table S1 in Supplement 2).

DBH. As shown in Table S2 in Supplement 2, virtually all eligible *DBH* studies measured activity in blood. We observed robust associations with enzyme activity for 2 *DBH* 5′ SNPs, rs1611115 (-1021C/T) and *DBH-STR,* as well as for an SNP in intron 5 (rs2519152) (Figure 3). *DBH*-STR (16) is a GT dinucleotide repeat polymorphism in the 5′ flanking region, with 12 and 13 repeats being most common. The effect of rs1611115 (-1021C/T) is especially notable, being observed in all 11 studies in which it has been assessed. There is no linkage disequilibrium between rs1611115 and rs2519152 (R^2 = .09), a finding that suggests that these polymorphisms are independently associated with DBH activity.

MAOA. The *MAOA* gene contains a 5′ variable number of tandem repeats (uVNTR) that has been extensively investigated. Most studies have compared groups designated "high" activity (the common 4-repeat allele, combined with the 3.5-repeat allele) and "low" activity (the common 3-repeat allele, combined with the 2-repeat and 5-repeat alleles) on the basis of an initial report (17). By meta-analysis, we confirmed that the uVNTR is associated with enzyme activity measured in blood or in brain (the latter using a positron emission tomography ligand) (Figure S4A in Supplement 1). Luciferase assays in cell lines are consistent with these data, showing greater activity for 4-repeat than 3-repeat allele (Table S3 in Supplement 2). The uVNTR does not influence *MAOA* mRNA abundance (Figure S4B in Supplement 1). Methylation has been assessed in 8 studies, with no uVNTR effects shown (Table S3 in Supplement 2).

MAOB. MAOB enzyme activity has been studied with regard to 2 intronic polymorphisms: a GT repeat variant and rs1799836. Most data are in platelets, and the studies show no

Table 1. Summary of Systematic Review Findings

Gene	Supplemental Table	Polymorphism[a]	Parameter (Tissue/Cell Type)	N[b]	Finding for Genotype[c]
COMT	S1	rs4680 (Val^{158}Met)	DNA methylation (mixed tissues)	9	Mixed: 5 show Met>Val; 2 show Val>Met; 2 show no difference
			mRNA abundance (mixed tissues)	9	No difference (Figure 2)
			Protein abundance (mixed tissues and cell lines)	10	Val>Met (Figure 2)
			Enzyme activity (mixed tissues, cell lines, and recombinant protein)	25	Val>Met (Figure 2)
			Enzyme stability (mixed tissues, cell lines and recombinant/in vitro translated protein)	12	Val>Met (Figure 2)
		rs165599	mRNA abundance (brain and lymphoblasts)	4	Mixed: 2 show reduced allelic expression of G vs. A; 1 shows lower expression of extended 3′ UTR transcript in A vs. G; 1 shows no genotype effect
		rs6269	Enzyme activity (liver and erythrocytes)	3	No effect (1 of 3 shows G>A, but doesn't control for strong LD with rs4680)
DBH	S2	DBH-STR	Enzyme activity (serum/plasma)	5	12R/12R>13R (Figure 3)
		rs1611115	Promoter activity (in vitro)	3	Mixed: 2 studies show C>T; 1 study shows T>C
			Enzyme activity (serum/plasma)	18	C>T (Figure 3)
		rs1108580	Enzyme activity (plasma/CSF)	3	2 studies show no effect in controls; results in psychiatric patients mixed
		rs2519152	Enzyme activity (serum/plasma)	5	G>A (Figure 3)
		rs1989787	Enzyme activity (plasma)	4	All show T>C
		rs6271	Enzyme activity (plasma)	6	Mixed: 2 show no effect; 2 show C>T; 1 shows T>C; 1 is a linkage study
		rs141116007 (5′-ins/del)	Enzyme activity (serum/plasma)	3	All show ins > del (but do not control for LD with other DBH SNPs)
MAOA	S3	uVNTR	DNA methylation (brain, lymphoblasts, blood, saliva, cell lines)	8	Mixed: 5 show no effect; 1 shows 3R/4R>4R/4R (women only); 1 shows 4R>3R; 1 shows genotype difference but direction of effect unclear
			Promoter activity (cell lines)	5	All show 3.5R and 4R>3R
			mRNA abundance (brain, blood, placenta, and cell lines)	8	No effect (Figure S4 in Supplement 1)
			Enzyme activity (brain, placenta, cultured fibroblasts)	5	"High activity" > "Low activity" (Figure S4 in Supplement 1)
MAOB	S4	Intron 2 (GT)n repeat	Enzyme activity (platelets)	3	3 studies: no effect
		rs1799836	Enzyme activity (platelets/plasma)	10	Most studies show no effect; 1 shows A>G; 1 shows G>A (Figure S5 in Supplement 1)
DAT	S5	rs28363170	Promoter activity (cell lines)	9	Mixed: 5 showed no difference between 9R and 10R; 3 showed 9R>10R (but was cell-type dependent); 1 showed 10R>9R
			mRNA abundance (brain, cell lines, iPSC-derived neurons)	7	No effect (Figure S6 in Supplement 1)
			DAT binding (striatum)	18	No effect (although significance reached if postmortem studies excluded) (Figure S6 in Supplement 1)
		rs3836790	DAT binding (striatum, cerebellum)	4	Mixed: 3 show no effects (in controls); 1 shows 5R>6R

Table 1. Continued

Gene	Supplemental Table	Polymorphism[a]	Parameter (Tissue/Cell Type)	N[b]	Finding for Genotype[c]
DRD2	S7	rs1800497 (Taq1A)	Receptor binding (striatum and extrastriate brain regions)	12	A2/A2>A1 (Figure S7 in Supplement 1)
		rs1799732 (-141ins/del)	Receptor binding (striatum and extrastriate brain regions)	6	No effects (Figure S7 in Supplement 1)
		rs6277 (C957T)	Receptor binding (striatum and extrastriate brain regions)	4	Mixed: 3 found CC<T carriers, but 1 was a publication of a second region in the same dataset; 1 found TT<C carriers
		rs1801028 (Ser311Cys)	Receptor binding (transfected cells and striatum)	5	Mixed: 3 showed no effects; 1 showed lower affinity for Cys allele; 1 showed no change in affinity but less D2S sequestration after dopamine
		rs1076560 (rs2283265)	mRNA abundance (frontal cortex)	6	Ratio of D2S/D2L GG>T (Figure S7 in Supplement 1)
		rs1079597 (Taq1B)	Receptor binding (striatum)	3	Mixed: 1 showed no effect; 2 showed B2>B1, but SNP is in strong LD with rs1800497
DRD3	S8	rs6280	Receptor binding (transfected cells and striatum)	6	Mixed: 4 show no effect; 2 show G>A affinity for dopamine
DRD4	S9	Exon 3 VNTR	Receptor binding (in vitro)	8	Mixed: 6 of 8 studies show no difference; 2 show 2R and 4R<7R spiperone and clozapine binding, but only in the presence of sodium chloride
		rs1800955 (-521CT)	Promoter activity (in vitro)	3	Mixed: 2 of 3 studies show no difference; 1 shows C>T
		5′ UTR 120-bp repeat	Promoter activity (in vitro)	4	Mixed: 1 study showed no effect; 3 showed reduced activity in association with duplication, but 2 were specific to cell type or conditions
TH	S11	rs10770141	Promoter activity (in vitro)	3	Mixed: 1 study shows no effect; 2 show T>C

3R, 3-repeat polymorphism; 3.5R, 3.5-repeat polymorphism; 4R, 4-repeat polymorphism; 5R, 5-repeat polymorphism; 12R, 12-repeat polymorphism; bp, base pair; CSF, cerebrospinal fluid; D2L, dopamine D_2 receptor long transcript isoform; D2S, dopamine D_2 receptor short transcript isoform; DAT, dopamine transporter; del, deletion; ins, insertion; iPSC, induced pluripotent stem cells; LD, linkage disequilibrium; mRNA, messenger RNA; SNP, single nucleotide polymorphism; UTR, untranslated region.

[a]Information is included for polymorphisms where at least three studies examined a given functional parameter.

[b]Number of studies included in systematic review.

[c]Findings from meta-analyses are indicated by reference to Figures and Supplemental Figures.

consistent genotype effects (Table S4 in Supplement 2). Three rs1799836 studies were suitable for meta-analysis, and they showed no effect of the SNP (Figure S5 in Supplement 1).

Dopamine Transporter (*DAT*): Weak Evidence for Functional Polymorphisms

In *DAT* studies, the most studied polymorphism is rs28363170 (usually referred to as the *DAT* 3′ VNTR), which exists as different-length alleles, most commonly 9- or 10-repeat alleles (18). Our meta-analysis found no association between the 3′ VNTR and *DAT* mRNA abundance (Figure S6A in Supplement 1). Associations between the 3′ VNTR and DAT binding were significant only at trend level (Figure S6B in Supplement 1). If the 2 postmortem studies are excluded, the genotype difference (10-repeat/10-repeat>9-repeat carriers) seen in the neuroimaging studies reaches nominal significance (Table 2). Results for this SNP in luciferase assays were variable (Table S5 in Supplement 2). Three other *DAT* SNPs (rs3836790 [intron 8 VNTR], rs6347, and rs27072) have all been examined in ≥5 studies, but no replicated functional correlates have been observed (Table S5 in Supplement 2).

Polymorphisms Are Associated With the Function of Dopamine D_2 Receptor but Not Other Dopamine Receptors

DRD1. We found 7 eligible studies of *DRD1*. There have been no replicated functional effects of any *DRD1* SNP, except rs686, which affected promoter activity in a luciferase assay in 2 different cell lines (Table S6 in Supplement 2).

DRD2. We included 27 studies of *DRD2* in the systematic review (Table S7 in Supplement 2), of which 14 studies, of 3 SNPs, were meta-analyzed. Of the included studies, 13 focused on rs1800497 (formerly known as the Taq1A site, located downstream of the 3′ end of *DRD2* in the adjacent *ANKK1* gene). This SNP was robustly associated with dopamine D_2 receptor (DRD2) binding (Figure S7A in Supplement 1). In contrast, we found no evidence for an association between the -141Ins/Del polymorphism (rs1799732) and DRD2 binding (Figure S7B in

Table 2. Summary of Meta-analytic Findings

Gene	SNP rs (Common Synonym)	Parameter	Number of Studies[a]	Number of Subjects[b]	Standardized Mean Difference (95% CI)[c]	Overall Genotype Effect, *Z*, *p*	Heterogeneity, I^2, % (*p* Value)
COMT	rs4680 (Val^{158}Met)	mRNA abundance	5 (5/0)	210 (141/69)	−0.17 (−0.47, 0.13)	1.13, .26	0 (.57)
		Protein abundance	6 (4/2)	257 (175/82)	−0.71 (−1.15, −0.27)	3.17, .002	53 (.06)
		Protein stability	3 (0/3)	358 (269/89)	−1.43 (−2.28, −0.58)	3.31, .0009	83 (.003)
		Enzyme activity	16 (5/11)	1307 (900/407)	−1.41 (−1.84, −0.97)	6.40, <.00001	88 (.00001)
DAT	rs28363170 (3′ VNTR)	mRNA abundance	3 (3/0)	66 (25/41)	−1.51 (−3.74, 0.71)	1.33, .18	91 (<.00001)
		Binding	12 (12/0)	523 (247/276)	0.22 (−0.04, 0.48)	1.68, .09[d]	47 (.03)
DBH	rs1611115	Enzyme activity	11 (0/11)	1017 (372/645)	−1.51 (−2.14, −0.89)	4.74, <.00001	94 (<.00001)
	DBH-STR	Enzyme activity	4 (0/4)	253 (159/94)	−0.70 (−1.20, −0.20)	2.73, .006	68 (.03)
	rs2519152	Enzyme activity	3 (0/3)	545 (360/185)	0.61 (0.24, 0.97)	3.28, .001	58 (.09)
DRD2	rs1800497 (Taq1A)	Binding	11 (11/0)	500 (204/296)	−0.52 (−0.72, −0.32)	4.99, <.00001	16 (.30)
	rs1799732 (-141InsDel)	Binding	5 (5/0)	243 (99/144)	0.15 (−0.29, 0.59)	0.68, .50	47 (.11)
	rs1076560 (rs2283265)	D2S/D2L mRNA	5 (5/0)	504 (141/363)	−1.20 (−2.03, −0.38)	2.86, .004	91 (<.00001)
MAOA	uVNTR	mRNA abundance	4 (2/2)	205 (80/125)	−0.18 (−0.74, 0.39)	0.62, .53	73 (.01)
		Enzyme activity	5 (3/2)	176 (68/108)	−0.42 (−0.74, −0.11)	2.67, .008	0 (.51)
MAOB	rs1799836	Enzyme activity	3 (0/3)	642 (238/304)	−0.09 (−0.55, 0.36)	0.40, .69	71 (.03)

CI, confidence interval; D2L, dopamine D_2 receptor long transcript isoform; D2S, dopamine D_2 receptor short transcript isoform; mRNA, messenger RNA; SNP, single nucleotide polymorphism; VNTR, variable number of tandem repeats.

[a]Number of studies included in meta-analysis. In parentheses is shown the number that used brain tissue followed by the number that used other tissues (e.g., plasma, erythrocytes).

[b]Total sample size. In parentheses is shown the number of rare allele carriers followed by the number of common (ancestral) allele homozygotes.

[c]Calculated using a random effects approach (see Methods and Materials).

[d]If postmortem studies are omitted, standardized mean difference (95% CI) = 0.28 (0.01, 0.55), $Z = 2.03$, $p = .04$.

Supplement 1) or other parameters. rs1800497 and rs1799732 are not in linkage disequilibrium ($R^2 = .009$). In addition, we found an association between rs1076560, an intronic SNP, and the relative abundance of the DRD2 short and long transcript isoforms (D2S and D2L) in the prefrontal cortex (Figure S7C in Supplement 1). This SNP is in moderate linkage disequilibrium with rs1800497 ($R^2 = .53$) but not with rs1799732 ($R^2 = .006$).

Other well-studied *DRD2* polymorphisms, rs6277 (C957T) and rs1801028 (Ser311Cys), could not be meta-analyzed. Our qualitative data synthesis for these SNPs showed no consistent functional evidence for rs6277, and we found some evidence that rs1801028 influenced the response of the DRD2 to agonist stimulation in vitro (Table S7 in Supplement 2).

DRD3. We found only 1 SNP in *DRD3* that has been studied for functionality: rs6280 (Ser9Gly). Findings are inconsistent or negative (Table S8 in Supplement 2).

DRD4. A total of 30 *DRD4* studies were eligible for qualitative synthesis, but no meta-analyses were possible (Table S9 in Supplement 2). The most studied *DRD4* polymorphism is a 48bp VNTR in exon 3, which exists as 2 to 11 repeats; interest was stimulated by a report suggesting that this VNTR influences clozapine binding (19). We found several reports of associations between the VNTR and DRD4 ligand binding and protein interactions; however, many results are negative, and no consistent picture has emerged. We reached similar conclusions regarding rs1800955 (-521C/T), a 5′ untranslated region 120–base pair repeat polymorphism, and regarding the other *DRD4* SNPs investigated (Table S9 in Supplement 2).

DRD5. We found 3 eligible *DRD5* studies, each of which studied a different *DRD5* SNP (Table S10 in Supplement 2). No functional effects were reported.

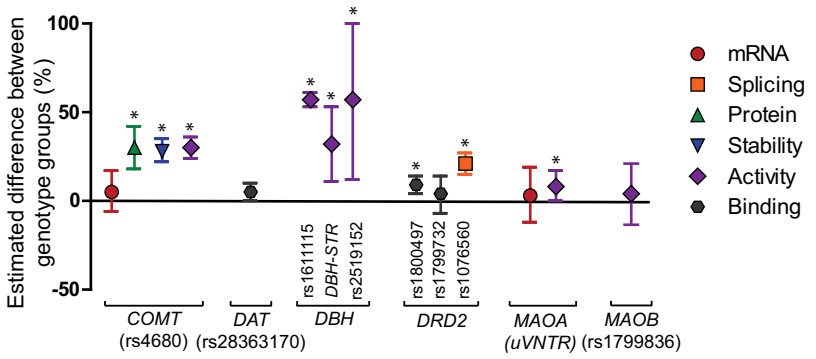


Figure 1. Estimated magnitude of effect of the various dopamine single nucleotide polymorphisms on different aspects of gene product function. The impact of the *DAT* rs28363170 single nucleotide polymorphism on *DAT* messenger RNA (mRNA) abundance is not shown, as the group difference could not be reliably estimated (see Methods and Materials). The colors shown are consistent across subsequent Figures and Supplemental Figures. *Genotype effect reaches statistical significance by meta-analysis ($p < .05$). uVNTR, 5′ variable number of tandem repeats.

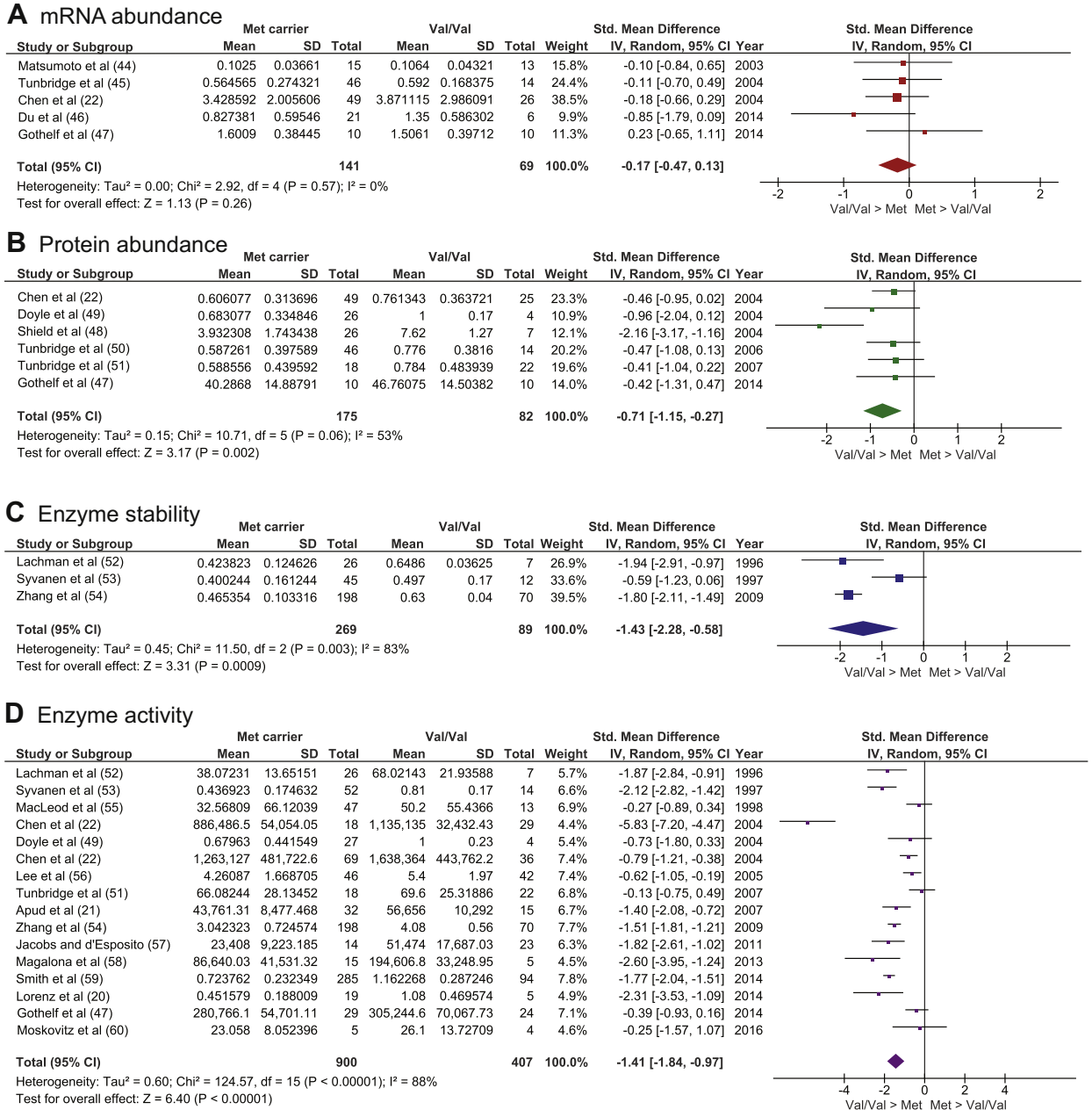


Figure 2. Forest plots for random effects meta-analyses of the association between the *COMT* rs4680 (Val[158]Met) polymorphism and **(A)** messenger RNA (mRNA) abundance, **(B)** protein abundance, **(C)** protein stability, and **(D)** enzyme activity. CI, confidence interval; IV, inverse variance. [Data from (20–22,44–60).]

No Consistent Evidence for Functional Polymorphisms in Dopamine Synthetic Enzymes or Vesicular Transporters

TH. We found 7 eligible *TH* studies, in which functional effects of 7 *TH* polymorphisms were investigated. Qualitative synthesis showed that findings were inconsistent, were not replicated, or lacked statistical evidence to substantiate reported genotype differences (Table S11 in Supplement 2).

DDC. No eligible studies investigating functional effects of polymorphisms in *DDC* were identified.

VMAT1. Only 2 eligible studies of *VMAT1* were identified, both in cell lines, and for each, investigators reported a functional correlate of a SNP (Table S12 in Supplement 2).

VMAT2. In all, 5 eligible studies were identified. In 2 of these, investigators reported effects of different SNPs on promoter

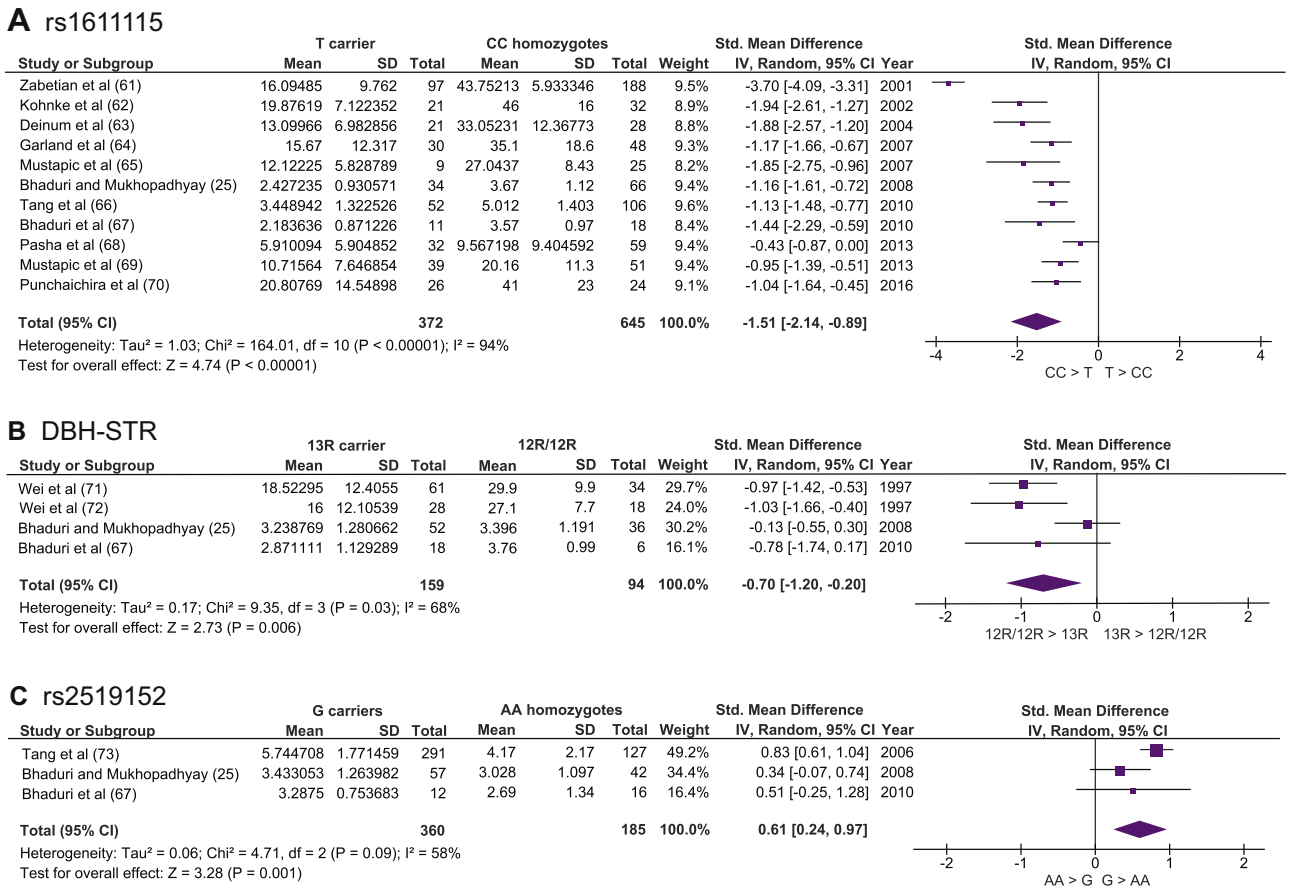


Figure 3. Forest plots for random effects meta-analyses of associations between **(A)** rs1611115, **(B)** *DBH*-STR, and **(C)** rs2519152 polymorphisms and dopamine β-hydroxylase activity. 12R, 12-repeat polymorphism; 13R, 13-repeat polymorphism; CI, confidence interval; IV, inverse variance. [Data from (25,61–73).]

activity; in 1, investigators described an SNP effect on mRNA abundance (Table S13 in Supplement 2).

Secondary and Sensitivity Analyses

Allele dose effects for *COMT* Val^{158}Met have been reported in some studies. We investigated this meta-analytically, comparing heterozygotes against each homozygote. All comparisons were statistically significant for protein abundance, protein stability, and enzyme activity (Figure S8 in Supplement 1), confirming an allele dose-dependent relationship between the polymorphism and these parameters (i.e., Val/Val>Val/Met>Met/Met). Allele dose effects were also obtained for associations with DBH activity for both rs1611115 and rs2519152 (Figure S9A in Supplement 1). For *DBH*-STR, there was an allele dose-dependent relationship with DBH activity, but the comparison between the heterozygote and minor allele group reached only trend-level significance (Figure S9B in Supplement 1).

We intended to investigate whether gender influenced *COMT* functional associations (15) but found only 3 studies (of enzyme activity) that allowed this issue to be addressed (20–22). None provided any indication for a gender difference in the functional effect of the Val^{158}Met genotype.

Table 2 shows that many of the meta-analytic results showed significant heterogeneity. We explored possible reasons for this finding, in concert with inspection of the funnel plots (Figure S3 in Supplement 1). For example, whether the heterogeneity reflected greater effects in one tissue type than another, or methodological differences in how a given functional parameter was measured. However, we found no clear nor consistent explanations. In meta-analyses where 1 study showed a markedly different effect size than the others, we repeated the meta-analysis after omission of this study; in each case, the result remained significant (data not shown).

DISCUSSION

Many studies report statistical associations between polymorphic variation in dopamine genes and brain phenotypes such as cognition, emotion, neuroimaging parameters, clinical status, and drug responses. However, in many cases, there is little or no evidence that the variant is functional—the null hypothesis is that a polymorphism is not—and hence, in isolation, a genotype-phenotype association is insufficient evidence to establish functionality. Empirical evidence is also required to demonstrate the existence and nature of a variant's functional effect—put

simply, whether it increases or decreases gene function. To address this issue, we performed systematic reviews and meta-analyses of the evidence for functionality of dopamine gene variants. We thereby demonstrate robust positive associations between polymorphisms in *COMT*, *DBH*, *MAOA,* and *DRD2* and the function of their gene products. In contrast, evidence for functional effects of the well-studied 3′ VNTR polymorphism in *DAT* was equivocal, and evidence for the genes that encode other dopamine receptors, synthetic enzymes, and vesicular transporters was inconsistent, negative, or absent. Our findings are summarized in Tables 1 and 2, with the magnitudes of meta-analytic effects illustrated in Figure 1.

The clearest functional associations were for the *COMT* Val^{158}Met polymorphism (rs4680). The Met^{158} allele is strongly associated with lower abundance, stability, and activity of the COMT enzyme, compared with the Val^{158} allele. There is no effect on *COMT* mRNA, suggesting that the Val^{158}Met-related activity differences do not induce compensatory changes in gene expression. The positive associations are seen across all tissues studied, including brain. The magnitude of effect is approximately 30% for all 3 parameters affected by Val^{158}Met. Taken together, these findings are consistent with the evidence indicating that the polymorphism directly influences protein function by altering its thermal stability: the Met^{158} substitution results in a more thermolabile isoform (23) that, in turn, leads to reduced abundance, stability, and activity compared with that of the ancestral Val^{158} allele.

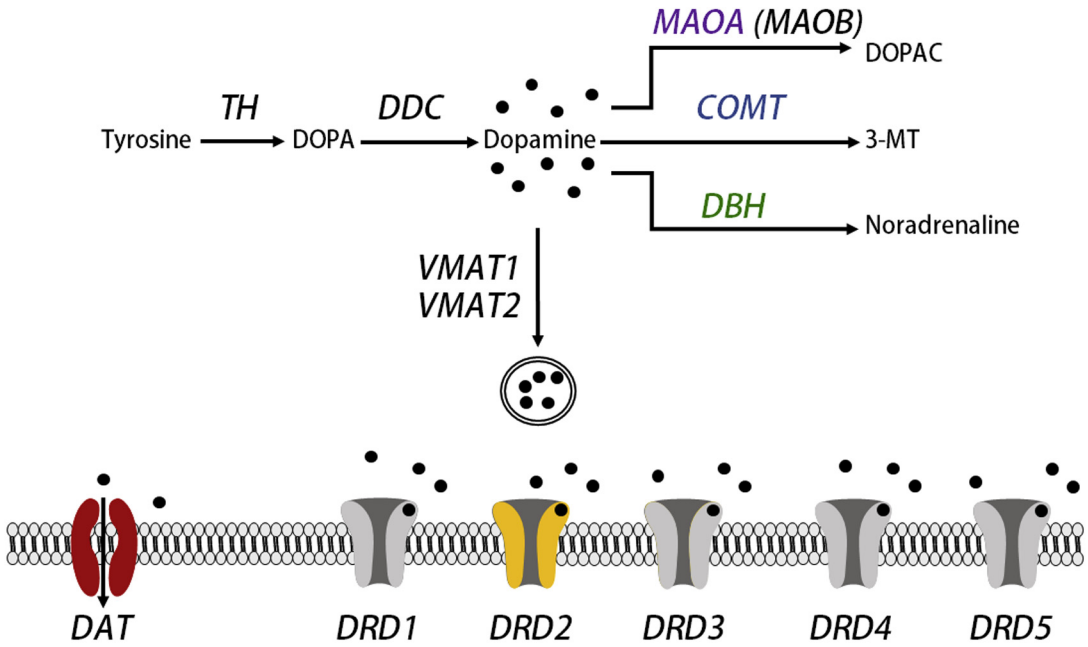
Functional polymorphisms were also observed in the other 2 dopamine catabolism genes. The *MAOA* uVNTR polymorphism has a relatively small (approximately 8%) but statistically robust association with enzyme activity. We found polymorphisms with large (approximately 30%–60%) effects on circulating DBH activity; the strongest association was for rs1611115, which has also been associated with DBH activity in a genome-wide association study (24). In addition, we identified an association with rs2519152, which was independent of the rs1611115 association, and a weaker association with *DBH*-STR (which is in moderate linkage disequilibrium with rs1611115) (25). These findings suggest that at least 2 loci in *DBH* influence its activity. However, all studies to date have focused on peripheral DBH activity; thus, their significance for brain DBH—and consequently for brain dopamine function—is unknown.

In the case of *DRD2*, 2 polymorphisms showed associations with distinct aspects of DRD2 function. Firstly, a modestly sized (approximately 9%) effect of the Taq1A polymorphism (rs1800497) on DRD2 binding. This association was strikingly consistent, including between in vivo and ex vivo studies. Our findings corroborate an earlier meta-analysis (26); however, our results are based on a significantly larger sample. Secondly, we found association between rs1076560 and the relative proportions of D2S and D2L splice isoforms, with the T allele associated with an approximately 20% reduction in the relative abundance of D2S. The isoforms encode DRD2s that show differential coupling to downstream effector pathways (27,28). D2S receptors were initially thought of as presynaptic and D2L receptors as postsynaptic (29). While true to an extent, this distinction has proved overly simplistic (30,31). Notably, the studies focused on DRD2 mRNA expression in the prefrontal cortex and striatum, so it is unknown whether rs1076560 influences D2S or D2L abundance in dopaminergic projection neurons.

A surprising aspect of our results is that the association between the well-studied *DAT* 3′ VNTR (rs28363170) and DAT binding was equivocal, particularly given that a clear positive association was previously reported, at least in studies using single photon emission computerized tomography (32). This difference may be due in part to our inclusion of postmortem radioligand binding studies, since the association reaches nominal significance if these are excluded. However, of greater significance, the earlier meta-analysis (32) considered DAT binding from multiple regions from the same individuals as separate datasets, thereby inflating the number of participants and overweighting the findings of these studies. Taken together, our equivocal findings for DAT binding, the small magnitude of effect (~5%), and the lack of an association with *DAT* mRNA expression argue against a major role for the 3′ VNTR in influencing DAT function.

With the exception of the *COMT* Val^{158}Met polymorphism, the molecular mechanisms underlying the positive associations between the polymorphisms examined here and the function of their gene products are unknown. All are noncoding (apart from rs1800497), being in upstream, downstream, or intronic regions. We found little evidence that they mediate their effects by simply altering total mRNA abundance. Thus, while rs1611115 has been examined with respect to *DBH* promoter activity and mRNA abundance, the presence and direction of effect varies between studies. Similarly, our findings suggest that the *MAOA* uVNTR is not associated with *MAOA* expression. While some of this disconnect may be due to technical factors (e.g., the tissue studied), it is also possible that some functional polymorphisms mediate their effect by altering more subtle aspects of gene function, such as alternative promoter usage or differential splicing. Given its effect on the relative expression of DRD2 splice isoforms, rs1076560 (or an SNP in linkage disequilibrium with it) likely influences a cis regulatory splicing signal and consequently the inclusion or exclusion of the adjacent exon 6 (33). For the other genes, the potential for isoform-specific effects are less clear: there are only 2 annotated human *MAOA* transcripts and a single *DBH* transcript (34). However, recent findings demonstrate that current human brain transcript annotations are far from complete (35), suggesting transcript-specific polymorphic effects as an avenue for future research.

In Figure 4, we attempt to synthesize what we can conclude from our results, and, importantly, what remains unknown. In essence, although the meta-analytic findings identify several functional polymorphisms in dopamine genes, predicting their individual or cumulative effect on dopaminergic function is not straightforward. For example, although the *COMT* and *MAOA* polymorphisms influence enzyme activity across all tissue and cell types studied, their significance likely varies between brain regions because of differences in the relative importance of enzymatic degradation versus reuptake for dopamine clearance. Specifically, while reuptake is the predominant mechanism in the striatum, enzymatic degradation (particularly by COMT) predominates in the cortex (36); therefore, *COMT* and *MAOA* functional polymorphisms are likely to be of markedly greater significance in the cortex than in the striatum, and vice versa for *DAT*. Nevertheless, alleles associated with lower activity of the products of these



Gene	SNP	Predicted effect on dopamine signalling	Key brain region affected
MAOA	uVNTR	↑ (3R > 4R + 3.5R)	Cortex
COMT	rs4680 (Val^{158}Met)	↑ (A [Met] > G [Val])	Cortex
DBH	rs1611115	Unclear	Unknown
	DBH-STR	Unclear	Unknown
	rs2519152	Unclear	Unknown
DRD2	rs1800497 (Taq1A)	Unclear	Striatum (effects elsewhere unclear)
	rs1799732 (-141InsDel)	Unclear	Striatum (effects elsewhere unclear)
	rs1076560 (rs2283265)	Unclear	Prefrontal cortex (effects elsewhere unclear)
**DAT*	rs28363170 (3′ VNTR)	*↑ (9R > 10R/10R)	Striatum

*Only significant at trend level by meta-analysis

Figure 4. The predicted effect of functional polymorphisms on dopamine signaling. The upper panel highlights (in color) the genes in the dopamine signaling pathway that contain functional polymorphisms; genes with no identified functional polymorphisms are shown in gray. The lower panel summarizes, where possible, each polymorphism's predicted effect on dopamine signaling and the brain regions in which these effects likely pertain. 3-MT, 3-methoxytyramine; 3R, 3-repeat polymorphism; 3.5R, 3.5-repeat polymorphism; 4R, 4-repeat polymorphism; 9R, 9-repeat polymorphism; 10R, 10-repeat polymorphism; DOPA, dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; InsDel, insertion or deletion; SNP, single nucleotide polymorphism; uVNTR, 5′ variable number of tandem repeats.

genes are broadly predicted to result in greater dopamine signaling in the relevant brain region. In contrast, the effects of *DBH* functional polymorphisms on dopamine signaling are unknown, since it is unclear whether they influence central dopamine levels and, if so, which brain regions are affected. Similarly, the net effect of the *DRD2* functional polymorphisms on dopamine signaling is difficult to predict, given the dual role of DRD2 as an inhibitory autoreceptor and a postsynaptic receptor (37,38). Beyond the issues relating to individual genes, the effects of polymorphisms are superimposed on the complexities of the dopamine system, which include distinct signaling modes operating at different time scales (39) and reciprocal relationships between dopamine signaling in different brain regions (40). Thus, we advise those using these polymorphisms as markers of central dopamine function not to attempt to combine multiple polymorphisms into a single measure using overly simplistic approaches.

It is notable that most functional dopaminergic polymorphisms are clustered in genes involved in dopamine clearance. *COMT*, *DBH,* and *MAOA* all contain polymorphisms that are associated with enzyme activity, and in the case of *COMT* and *DBH*, the magnitude of the genotype effect is large (>30%). In contrast, evidence for the presence of functional polymorphisms in the genes encoding synthetic enzymes or dopamine receptors, with the exception of *DRD2*, is lacking. Even in the case of *DRD2*, the polymorphisms' effects are

nuanced: the rs1800497 (Taq1A) polymorphism has only a small effect on DRD2 binding, and while rs1076560 has a larger effect, it affects a relatively subtle phenotypic measure (the relative abundance of *DRD2* mRNA splice isoforms, which may or may not affect the abundance of the encoded proteins). The notion that genetic modulation of dopamine function might be mediated primarily through regulating its breakdown and clearance is an appealing one. Given dopamine's key role in multiple aspects of brain function, it is likely that polymorphisms that drastically disturb global dopamine synthesis or availability would be selected against. For example, although TH activity is intricately regulated (41), disturbances to this regulation due to polymorphic variants are poorly tolerated. Thus, reduced TH activity due to heterozygous missense mutations in the gene results in a profound clinical phenotype, and no homozygous or compound heterozygous mutations have been identified (42). In contrast, polymorphisms that subtly alter receptor function, or affect dopamine breakdown, potentially provide a means to fine-tune the response to released dopamine. Furthermore, the modulation of response rather than synthesis might allow for some degree of spatial (and perhaps temporal) selectivity in a polymorphism's effect, given regional variation in the relative abundance of different dopamine receptors and the prominence of reuptake versus breakdown clearance mechanisms.

A striking aspect of our findings is that in many cases (notably for *MAOA* and *DBH*), data from in vitro studies were mixed or failed to predict in the in vivo situation. While this may point to cell type–specific effects, it illustrates the need to exercise caution when generalizing from in vitro findings in the absence of corroborative evidence that a polymorphism is or is not functional. This caution applies even more so to predictions made in silico.

Experiments examining associations between a polymorphism and gene function can only ever provide positive evidence; they cannot prove absence of functionality. The studies that were amenable to meta-analysis tended to focus on "global" measures of function, such as total gene expression, receptor binding, and enzyme activity. Therefore, as noted above, we cannot preclude effects on more specific phenotypic measures or on functional effects that are dependent on demographic factors such as age or gender, or that manifest only under specific physiological conditions, e.g., stress. Furthermore, polymorphisms that in isolation are not functional may form part of functional haplotypes resulting from nonlinear, interactive effects between multiple polymorphisms. For example, in the case of COMT, in addition to directly influencing COMT activity, $Val^{158}Met$ also forms part of a haplotype that alters the secondary structure of the resulting RNA and thence affects enzyme activity (43). Ultimately, the strength of the evidence required to exclude a given polymorphism from being of interest will depend on the research question under investigation. The significant heterogeneity found in some of the meta-analyses provides another note of caution when interpreting the results.

Conclusions

The aim of this study was to assess which polymorphisms might serve as useful indices of central dopamine function in human studies. We demonstrate that polymorphisms in *COMT*, *MAOA*, and *DRD2* provide information about biologically relevant measures of the function of their gene products in the human brain.

We argue that studies linking individual polymorphisms to brain phenotypes are most valuable when driven by clear hypotheses based on knowledge of the underlying neurobiology. The dopamine system is not a unitary construct; instead, dopamine signaling operates over several distinct time courses and across separable but interconnected projection systems. The various functional polymorphisms likely contribute to different aspects of dopamine signaling, spatially and temporally, both because of the brain regions and cell types in which the genes themselves are expressed and because of the inherent complexities of the dopamine systems. Thus, we advise against the "dopamine scoring" approach, in which the effects of different polymorphisms are assumed to be additive and are linearly combined. We do not advocate focusing exclusively on the neural correlates of polymorphisms whose functionality is well established; however, other things being equal, we would argue that associations are a priori more plausible for polymorphisms that are of unequivocal functional significance. Although we focused on dopamine, these issues pertain to all genes, and we therefore encourage researchers to appraise critically the evidence for functionality of all polymorphisms in which they are interested.

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