Transcriptomic context of *DRD1* is associated with prefrontal activity and behavior during working memory

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Dopamine D_1 receptor (D_1R) signaling shapes prefrontal cortex (PFC) activity during working memory (WM). Previous reports found higher WM performance associated with alleles linked to greater expression of the gene coding for D_1Rs (*DRD1*). However, there is no evidence on the relationship between genetic modulation of *DRD1* expression in PFC and patterns of prefrontal activity during WM. Furthermore, previous studies have not considered that D_1Rs are part of a coregulated molecular environment, which may contribute to D_1R-related prefrontal WM processing. Thus, we hypothesized a reciprocal link between a coregulated (i.e., coexpressed) molecular network including *DRD1* and PFC activity. To explore this relationship, we used three independent postmortem prefrontal mRNA datasets (total $n = 404$) to characterize a coexpression network including *DRD1*. Then, we indexed network coexpression using a measure (polygenic coexpression index—*DRD1*-PCI) combining the effect of single nucleotide polymorphisms (SNPs) on coexpression. Finally, we associated the *DRD1*-PCI with WM performance and related brain activity in independent samples of healthy participants (total $n = 371$). We identified and replicated a coexpression network including *DRD1*, whose coexpression was correlated with *DRD1*-PCI. We also found that *DRD1*-PCI was associated with lower PFC activity and higher WM performance. Behavioral and imaging results were replicated in independent samples. These findings suggest that genetically predicted expression of *DRD1* and of its coexpression partners stratifies healthy individuals in terms of WM performance and related prefrontal activity. They also highlight genes and SNPs potentially relevant to pharmacological trials aimed to test cognitive enhancers modulating *DRD1* signaling.

gene coexpression network | DRD1 | working memory | fMRI | polygenic score

Decades of exploration of the relationship between dopamine (DA) and cognition have established a crucial role of prefrontal D_1 receptors (D_1Rs) in working memory (WM) (1–8). D_1Rs modulate prefrontal synaptic neurotransmission by mediating recurrent excitation in local circuits, which is considered a key WM mechanism (3, 9, 10). Furthermore, increased D_1R signaling enhances the signal-to-noise ratio in prefrontal neuronal networks (5) and contributes to improved WM performance in nonhuman primates (11). Further evidence also suggests that extreme hypo- or hyperstimulation of D_1Rs is detrimental to prefrontal physiology related to WM and predicts poorer WM performance (1, 6, 12, 13). Taken together, these results suggest that the level of D_1R signaling in the prefrontal cortex (PFC) is important for efficient WM processing. In particular, functional imaging studies in humans have considered lower prefrontal activity paralleled by higher or unaffected WM performance as a correlate of efficient WM processing (14).

Notably, WM behavior and related prefrontal activity have an estimated heritability around 0.4 (15–17), and the heritable component of WM is associated with DA-related genetic variation (18). Given the primary role of D_1R signaling in WM, it has been hypothesized that, across individuals, differences in WM behavior and brain activity may be related to the genetics of D_1R signaling. Consistently, functional single nucleotide polymorphisms (SNPs) of the gene coding for D_1Rs (*DRD1*, located in the 5q35.2 region in chromosome 5) have been associated with cognition and executive function. For example, previous studies have reported that the A allele of *DRD1* rs686 predicts greater in vitro *DRD1*expression (18–20). Another *DRD1* SNP, rs5326, has been associated with gene expression in the temporal gyrus and with *DRD1* transcriptional activity in human neuroblastoma cells (19, 21). Additionally, the A allele, associated with lower *DRD1* expression, predicted poorer executive function estimates. Overall, this evidence suggests that *DRD1* functional genetic variants may explain part of the interindividual variability in WM performance. These findings

Significance

Dopamine D_1 receptors in the prefrontal cortex (PFC) are critical for working memory (WM). However, it is unknown how D_1-related genetic background mediates differences in WM performance between humans. Furthermore, previous studies did not consider that *DRD1* is likely part of a coregulated molecular network, which may contribute to WM performance and its underlying neural correlates. The key of this research is the identification of a relationship between genetically predicted coexpression and WM processing. In particular, genetically predicted greater *DRD1*-related coexpression was associated with lower PFC activity and higher WM performance, indicating greater WM efficiency. Our findings may help to link gene expression with brain activity and to develop WM-enhancing drugs by differentiating individuals based on their genetic background.

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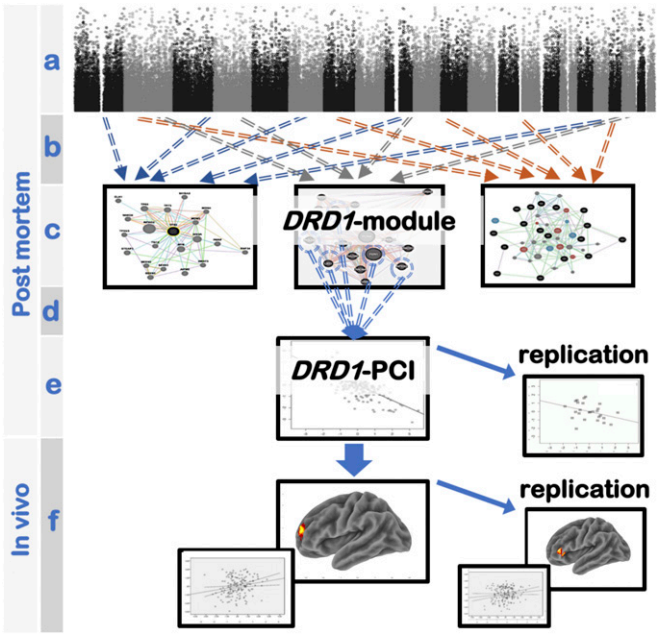

Fig. 1. Concept illustration of the study design. (*A*) Genome-wide genotyping; (*B*) Weighted Gene Coexpression Network Analysis identified modules of coexpressed genes; (*C*) prioritization of the coexpression module including *DRD1* (*DRD1*-module); (*D*) selection of the genes most associated with *DRD1* expression within the *DRD1*-module (*DRD1* gene set); (*E*) computation of a *DRD1* polygenic coexpression index, using genes within the *DRD1* gene set (*DRD1*-PCI); (*F*) association of *DRD1*-PCI with working memory performance and related brain activity.

also indicate that alleles associated with greater *DRD1* expression predict higher cognitive performance. However, previous works have fallen short of relating predicted gene expression of *DRD1* in the PFC with WM because the association between genetic variation and *DRD1* expression has not been studied in the human PFC. Furthermore, there is no evidence about the relationship between genetically determined *DRD1* expression and PFC activity during WM in humans.

Notably, *DRD1* has never been studied in the context of gene networks. Genes are expressed in coregulated networks (20), but little is currently known on (*i*) the interplay between *DRD1* and other genes in terms of gene expression regulation and (*ii*) the link between coregulated networks of genes including *DRD1* and prefrontal activity during WM. Therefore, the investigation of gene coexpression networks may yield more information and predictive power compared with those obtained by focusing on *DRD1* per se (20, 21). Additionally, recent studies have shown that combining multiple SNPs affords greater power on gene expression prediction studies compared with single SNP studies (22, 23).

On this basis, our aim was to identify a coexpression gene set including *DRD1* in the PFC and the genetic variants associated with the coexpression of the *DRD1*-related gene set. Furthermore, we investigated how genetically predicted coexpression of the *DRD1* gene set is associated with prefrontal WM processing. To this purpose, we refined an approach we have reported in a previous proof-of-concept work (21) (Fig. 1). Briefly, in the present work, after identifying a transcriptome-wide coexpression network in postmortem human dorsolateral PFC (DLPFC) (Brodmann area 46), we selected the coexpression gene set including *DRD1*. Then, we identified SNPs associated with coexpression [coexpression quantitative trait loci (coeQTLs)] of such a gene set. Thus, we combined the effect of these SNPs on coexpression into an index (polygenic coexpression index—*DRD1*-PCI) predicting the coexpression of the *DRD1*-related gene set. Finally, we tested whether the *DRD1*-PCI was linked with interindividual variation in PFC activity and behavior during WM in two independent samples of healthy individuals who participated in an N-back fMRI study. Based on previous studies (14, 18, 19), we expected that alleles predicting coexpression of the *DRD1* network and greater *DRD1* expression would also be associated with higher WM performance and lower PFC activity estimates, i.e., greater PFC efficiency. To account for potential effects of *DRD2* on the target phenotypes, we included our previously published *DRD2*-PCI in the fMRI and behavioral analyses.

Results

Weighted Gene Coexpression Network Analysis and Parceling. We used a previously reported gene coexpression network (21), based on the public dataset Braincloud (24) (Tables 1 and 2) of transcriptome-wide dorsolateral PFC gene expression. Using a newly developed technique (25), we parceled the very large Weighted Gene Coexpression Network Analysis (WGCNA) module that included *DRD1* (2,452 genes) to identify a more focused coexpression gene set related with *DRD1* expression. The parceling procedure detailed the *DRD1* transcriptomic context, and we obtained a smaller coexpression set of 126 genes (*SI Appendix*, Table S1). We replicated the gene set structure using the BrainEAC dataset (26) and the RNA sequencing dataset published by the CommonMind Consortium (CMC) (27) (Tables 1 and 2) and found that these genes were more strongly connected than chance (BrainEAC $P = 0.0037$; CMC $P < 0.0001$) (*SI Appendix*, Fig. S1*C*). Additionally, we found that, in BrainEAC, 85 of 126 genes contributed to the first principal component of gene expression (module eigengene) in the same direction observed in Braincloud, a higher-than-chance proportion (binomial test, $P = 5.7 \times 10^{-6}$). In CMC, 76 of 106 genes covaried just as observed in Braincloud (binomial test, $P = 1.6 \times 10^{-6}$). Therefore, both gene connectivity and the direction of gene coexpression was largely preserved in two independent datasets. Neither the large module nor the restricted gene set included the *DRD2* gene, and we found no significant correlation between *DRD1* and *DRD2* ($n = 199$, Spearman's $\rho = 0.12$, $P = 0.09$). None of the genes in the *DRD1* set were in the *DRD2* module we previously published (21).

We investigated the biological functions of this gene set and found that protein binding genes (88 hits, 1.38-fold enrichment, Bonferroni corrected $P = 0.021$) and genes functionally associated with DNA-dependent ATPase activity (6 hits, 12-fold enrichment, Bonferroni corrected $P = 0.036$) were overrepresented. The first principal component of gene set expression (gene set eigengene, GSE), which we used as a coexpression measure, explained 50% of the gene set expression variance. *DRD1* belonged to a minority of 47 genes negatively correlated with the GSE (*SI Appendix*, Fig. S1*B*) ($R^2 = 0.38$, $P < 2.2\ 10^{-16}$), suggesting that *DRD1* expression was higher when the expression of the majority of its partner genes was lower.

***DRD1* Polygenic Coexpression Index Computation in Postmortem Tissue.** We first assessed whether SNPs in the genomic proximity of *DRD1* were associated with *DRD1* expression but found no significant hit (uncorrected $\alpha = 0.05$). Then, we identified SNPs associated with the GSE using previously reported methods (21). Out of 3,079 SNPs included in the coexpressed genes and their 100-kbp flanks, we obtained a set of 13 independent significant hits (Table 3 and *SI Appendix*, Table S3). The ensemble of the 13 SNPs selected and the genetic variants in full linkage disequilibrium with them ($R^2 = 1$) included more genetic regulatory elements than expected by chance in the DLPFC ($P = 0.03$) (28). We used these 13 SNPs to compute a

Table 1. Gene expression datasets used in the study

Datasets	Age	RIN	PMI	pH
BC (N199; 139♂; AA105)	32[20]	8.4[0.5]	30[15]	6.5[0.3]
BrainEAC (N26; 20♂; AA0)	62[17]	6.6[0.5]	43[28]	6.3[0.2]
CMC (N179; 115♂; AA34)	58[17]	8.1[0.6]	16[7]	6.6[0.3]

SDs are indicated between square brackets. AA, African American; BC, BrainCloud dataset; CMC, CommonMind Consortium dataset; N, sample size; PMI, postmortem interval in hours; RIN, RNA integrity number.

Table 2. fMRI and behavioral datasets used in the study

Datasets	Age	EHI	SES	IQ
f/b-DISC (N152; 78♂)	27[7]	0.75[0.4]	43[16]	108[12]
f-REP (N149; 76♂)*	28[7]	0.74[0.4]	41[17]	106[13]
b-REP (N193; 89♂)*	27[8]	0.74[0.4]	39[17]	106[12]

SDs are indicated between square brackets. b-REP, behavioral replication sample; EHI, Edinburgh Handedness Inventory; f/b-DISC, fMRI and behavioral discovery sample; f-REP, fMRI replication sample; IQ, intelligence quotient; SES, socioeconomic status.
*f-REP and b-REP are partially overlapping (123 individuals in common).

polygenic coexpression index (PCI) associated with *DRD1* gene set coexpression (*DRD1*-PCI) (*SI Appendix*, Table S2 reports the weights attributed to all genotypes of these 13 SNPs). Additionally, we performed leave-one-out cross-validation on the SNP selection to estimate the reliability of the *DRD1*-PCI as a proxy of gene coexpression. We found that the *DRD1*-PCI significantly predicted the GSE in subjects not included in the training set (R^2 = 0.024, P = 0.029) (*SI Appendix*, Fig. S2). Since *DRD1* was negatively correlated with the GSE, we reversed the *DRD1*-PCI to have it positively correlated with *DRD1* expression for the sake of simplicity of graphic representation (*SI Appendix*, Fig. S2). We also screened possible associations of the *DRD1*-PCI with confounding variables [age, sex, ethnicity, RNA integrity number (RIN), pH, postmortem interval] and found a significantly higher *DRD1*-PCI in Caucasians than in African Americans (independent t test, $|t_{198}|$ = 2.9, P = 0.004), likely because of the population stratification of allelic frequencies. Analysis of covariance (ANCOVA) on the cross-validated *DRD1*-PCI revealed no significant main effect of ethnicity on the GSE ($F_{1,195}$ = 0.44; P = 0.51). Instead, the main effect of the leave-one-out cross-validated *DRD1*-PCI remained significant ($F_{1,195}$ = 5.23; P = 0.023). There was no significant *DRD1*-PCI × ethnicity interaction on GSE ($F_{1,195}$ = 0.08; P = 0.77), suggesting no significant difference in the slope of the association across ethnicities. Indeed, the cross-validated *DRD1*-PCI was associated with the GSE both in African Americans and Caucasians with comparable effect sizes (Caucasians: R^2 = 0.03; African Americans: R^2 = 0.022) (*SI Appendix*, Fig. S3). The *DRD1*-PCI association with the GSE was replicated in the same direction in BrainEAC (R^2 = 0.12, one-tailed P = 0.041) (*SI Appendix*, Fig. S2) and in CMC (R^2 = 0.017, one-tailed P = 0.043) (*SI Appendix*, Fig. S2). Notably, the association was significant despite the differences between datasets (including ethnicity differences) (Tables 1 and 2) and preprocessing procedures.

Association Between the DRD1-PCI and WM Processing. We tested the association between the *DRD1-PCI* and WM processing (29) in a sample of 371 unrelated healthy adult Caucasian volunteers (Tables 1 and 2).

fMRI. A general linear model was used to investigate the association between *DRD1*-PCI and brain activity using both linear and quadratic terms of the *DRD1*-PCI. The quadratic term of the *DRD1*-PCI was included to assess the documented U-shaped dose–response function between *DRD1* stimulation and PFC activity (5). We also included as a covariate the linear and quadratic term of the *DRD2*-PCI (21, 30), to control for potentially confounding effects. We found a negative association of the linear term of the *DRD1*-PCI with blood oxygen level-dependent (BOLD) response in the left PFC [middle frontal gyrus—Brodmann Area 10: x, y, z = −29, 53, 24; F = 22.25; Z = 4.51; family-wise error (FWE)-corrected cluster extent = 7 voxels, ∼345 mm^3, whole brain FWE-corrected P = 0.006] (Fig. 2 *A* and *C*). There were no other significant effects or interactions involving the *DRD1*-PCI. Similarly, in the fMRI replication sample, we found a negative association between the *DRD1*-PCI and activity in the left PFC, using the same whole brain statistical threshold (middle frontal gyrus—Brodmann Area 46: x, y, z = −40, 34, 5; T = 4.41; Z = 4.26; FWE-corrected cluster extent = 6 voxels, ∼316 mm^3; whole brain FWE-corrected P = 0.02) (Fig. 2 *B* and *D*). These findings imply that the BOLD signal was lower in individuals with higher predicted *DRD1* expression, corresponding to lower predicted coexpression of the majority of the gene set.

Behavioral Results. We used differential WM accuracy [accuracy at the greater WM load minus accuracy at the lower WM load (Δ_{WM})] as a WM performance measure, as suggested by previous work (31). As in the fMRI study, we tested the association between *DRD1*-PCI and WM performance using both linear and quadratic terms of the *DRD1*-PCI and of the *DRD2*-PCI. In the discovery sample, the general linear model revealed a main effect of the linear term of the *DRD1*-PCI ($F_{1,144}$ = 6.3; P = 0.014; partial η^2 = 0.041) (Fig. 2*E*) (see the *SI Appendix* for other effects). The *DRD1*-PCI correlated positively with Δ_{WM}, indicating higher WM capacity with greater predicted *DRD1* expression. The association of the *DRD1*-PCI with Δ_{WM} was also significant in the same direction in the replication sample (t_{186} = 1.91; one-tailed P = 0.029; partial η^2 = 0.019) (Fig. 2*F*). Therefore, individuals with alleles associated with higher *DRD1* expression and lower coexpression of the gene set had lower BOLD activity and higher behavioral performance.

To investigate the potential relationship between *DRD1*-related physiology, prefrontal activity, and other components of the dopaminergic system, such as *DRD2*, we used mediation and moderation analyses. Briefly, the analyses revealed significant mediation of prefrontal BOLD in the relationship between the *DRD1*-PCI and Δ_{WM}, and such mediation was moderated by the *DRD2*-PCI. However, the effects detected in the discovery sample failed to replicate and are reported in *SI Appendix, Mediation Analysis*.

Discussion

Multiple lines of evidence suggest that D_1R signaling exerts a key effect on WM performance and WM-related brain activity. It has been hypothesized that *DRD1* genetic variation may affect D_1R signaling, e.g., by affecting *DRD1* expression (18, 19). However, to our knowledge, there is no evidence of association of *DRD1* genetic variation with gene expression in the PFC. More importantly, there are no previous studies addressing how genetic ensembles related to *DRD1* affect prefrontal function during WM. The *DRD1*-PCI that we generated in the present study is an index of predicted coexpression of a *DRD1*-related gene set in the PFC. We found that this polygenic index was associated with WM behavior and related PFC activity across independent samples. In particular, the greater the *DRD1*-PCI, the higher the differential accuracy and the lower the PFC response during the N-back. These relationships survived, even considering the effect of

Table 3. Ranked single nucleotide polymorphisms (SNPs) included in the computation of the *DRD1*-PCI

SNP (module gene)	Association with GSE	Association with CG	Association with *DRD1*
rs7487813 (***UBE2N***)	2.22×10^{-5}	0.003	0.009
rs2267844 (*SLC26A6*)	1.43×10^{-4}	0.013	0.007
rs663208 (*CCDC81*)	3.71×10^{-4}	0.013	0.031
rs17005918 (***SCOC***)	4.51×10^{-4}	0.001	0.002
rs13101217 (***SEC22A***)	5.35×10^{-4}	0.015	0.006
rs1859464 (*PCNX1*)	9.22×10^{-4}	0.707	0.002
rs2278214 (***MGAT4A***)	1.21×10^{-3}	0.009	0.001
rs7915524 (*FAM171A1*)	2.05×10^{-3}	0.207	0.256
rs12509826 (***SCOC***)	3.67×10^{-3}	0.004	0.189
rs10134399 (*XRCC3*)	4.26×10^{-3}	0.079	0.095
rs10906841 (*FAM171A1*)	4.52×10^{-3}	0.003	0.356
rs2306251 (*GAK*)	4.61×10^{-3}	0.118	0.12
rs11602122 (*TPCN2*)	4.83×10^{-3}	0.221	0.005

The association columns report the *P* values of the association. The expression of genes reported in bold font is negatively correlated with the gene set eigengene, as is also the case of *DRD1*. CG, closest gene; GSE, gene set eigengene.

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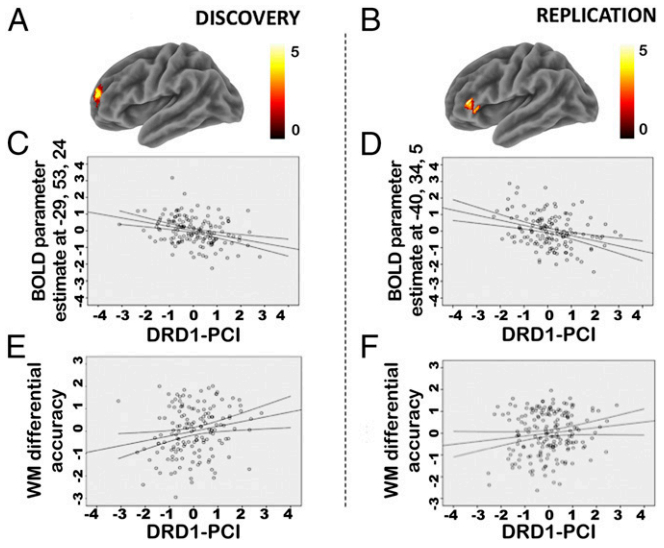

Fig. 2. Association of the *DRD1*-PCI with working memory-related prefrontal activity and working memory performance. (*A*) Rendering of the brain activity significantly associated in the discovery sample with *DRD1*-PCI in the dorsolateral prefrontal cortex, and color bar with the relative Z scores. (*B*) Rendering of the brain activity significantly associated in the replication sample with *DRD1*-PCI in the dorsolateral prefrontal cortex, and color bar with the relative Z scores. Left in the figures is left in the brain. (*C* and *D*) Scatter plots of the Z scores of the estimated activity in these clusters (*y* axis) as a function of the Z score of the *DRD1*-PCI. (*C*) Discovery sample; (*D*) replication sample. (*E* and *F*) Scatter plots show the Z score of the *DRD1*-PCI on the *x* axis and differential accuracy on the *y* axis. (*E*) Discovery sample; here the *y* axis reports the average of the two loads (Δ_{2-1} and Δ_{3-2}) after marginalization for covariates and standardization. (*F*) Replication sample; here, the *y* axis reports Δ_{2-1} after marginalization for covariates and standardization. Plots show trend lines and 95% confidence intervals of the mean.

another component of the dopaminergic system, *DRD2*, indexed by the *DRD2*-PCI. Based on previous models positing that greater PFC activity despite similar or lower behavioral proficiency is a correlate of less efficient WM processing (4, 7, 14), these results suggest that greater predicted *DRD1* expression and lower coexpression of the *DRD1*-related gene set are associated with greater WM efficiency.

The Genetic Architecture of DRD1 Transcription. The gene set we identified is reproducibly coexpressed, as shown by the replication in two independent datasets (26); further, this gene set seems independent of the transcriptomic context of *DRD2*. It is noteworthy that the majority of the genes in this set are down-regulated when *DRD1* is up-regulated. Gene ontology analysis revealed that genes related with DNA helicase activity were overrepresented in this set. Additionally, two of the SNPs that we identified as coeQTLs (rs7487813 and rs10134399) map close to genes in the coexpression gene set that are associated with DNA double-strand break repair (*UBE2N* and *XRCC3*). Both SNPs are associated, albeit weakly, with the expression of the respective genes (Table 3) (rs10134399 is associated with *XRCC3* in an independent study) (32). Double-strand DNA breaks have been highlighted as a mechanism linking neuronal activity with transcription regulation (33). Based on this activity–transcription relationship, the fact that *DRD1* expression covaries with that of genes related with double-strand DNA break repair may suggest a link between D_1R-related neuronal activity and transcription mechanisms of the *DRD1*-related gene set. Even if this contention is speculative based on the current results, relationships between transcription levels and brain activity are supported by the literature (34). The evidence we report may be used to select partner genes of *DRD1* as candidate genes for mechanistic investigations of the link between D_1R activity and *DRD1* gene set transcription levels in cell biology experiments.

The Role of Prefrontal D_1 Receptors in Working Memory. D_1R signaling supports WM-related persistent neural firing in the PFC (3, 10), allowing greater focus of neural resources on the task at hand (5). According to the model put forward by Seamans and Yang (5), D_1Rs enhance the signal-to-noise ratio in PFC neurons during WM performance by promoting activity in recurrent circuits. D_1Rs are thought to act via a specific block of task-unrelated excitatory inputs in the PFC (6, 10, 35). Consistently, small increases in D_1R signaling have been linearly correlated with WM behavior (10, 11, 36–38). Furthermore, previous findings indicated poorer WM performance in genetic or clinical conditions associated with reduced *DRD1* expression (19, 39, 40). Overall, the present results are consistent with these previous findings. In fact, greater predicted *DRD1* expression—as inferred based on its coexpression gene set—was associated with lower PFC activity, which has been consistently interpreted as a correlate of greater prefrontal efficiency during WM (14). Furthermore, greater predicted *DRD1* expression was also associated with higher WM behavioral accuracy. These findings also suggest that the regulation of *DRD1* expression is polygenic, rather than only based on genetic variants proximal to *DRD1*, and is embedded in the context of gene network coexpression.

Seamans and Yang (5) posited that excessive levels of D_1Rs impair WM performance (inverted-U model of WM function). Both animal (35) and human studies (41) support this interpretation. However, we found that the quadratic component of the PCI did not provide a significant fit either in brain or in behavioral phenotypes. It should be noted that we evaluated a genetic component associated with physiological variation in *DRD1* transcription levels in healthy individuals. Thus, a possible interpretation of our results is that we investigated variation within the physiological portion of the inverted-U curve—pharmacological stimulation or more extreme genotypic configurations may be necessary to observe the full inverted-U curve.

Limitations. The SNP set we used to compute the *DRD1*-PCI does not include variants that are significantly associated with coexpression per se because we used an uncorrected statistical threshold. Instead, we identified a set of 13 SNPs which, together, explain part of the coexpression of the *DRD1* gene set. Consistent with a putative role of the SNPs here identified as functional variants, previously reported regulatory variants of gene expression in the DLPFC were overrepresented in this SNP set. Further issues to be addressed are the different localization of the clusters identified in the discovery and replication samples and the lack of replication of the mediation and moderation analyses. Both the discovery and replication clusters were located in PFC regions relevant for WM (2). While relaxing the threshold revealed a substantial overlap between the clusters ($P < 0.05$, x, y, z = −40, 12, 9; Z = 2.28; k = 37), sample characteristics and study protocols may explain the difference in spatial localization when more stringent statistical thresholds are used. It remains noteworthy that the effect of the *DRD1*-PCI was significant in the PFC in the same direction in two independent samples, despite the different protocols employed.

Conclusions

The present results shed light on the understanding of the genetic modulation of WM-related phenotypes via D_1Rs and their transcriptomic context. Since the functional variants in multiple genes coexpressed with *DRD1* allow in vivo prediction of *DRD1* expression in the DLPFC, they may be relevant to clinical conditions associated with dysregulation of this gene (40). For example, clinical trials using D_1R agonists on patients with schizophrenia failed to demonstrate amelioration of cognitive symptoms (42). Thus, our findings may help in developing WM-enhancing drugs for clinical populations, acting on D_1Rs (42, 43), in a personalized medicine framework. Such an approach could be especially relevant for clinical conditions characterized by pervasive WM deficits like dementia, schizophrenia, and bipolar disorder (44–46).

Materials and Methods

Gene Coexpression Network Analysis in Postmortem PFC. We used Braincloud (24) (Tables 1 and 2) and Weighted Gene Coexpression Network Analysis (WGCNA) to obtain a transcriptome-wide coexpression network in the postmortem DLPFC of 199 individuals free of psychiatric and neurologic diseases. We used the same transcriptome-wide coexpression network published in our previous work (21), with the same subjects, without identifying a new network. Briefly, in our previous work, we selected postnatal samples from Caucasian and African American subjects with RNA integrity (RIN) >7.0, to ensure high data quality (47), and preprocessed the data to reduce the effect of confounders [demographical variables (i.e., age, sex, ethnicity) and sample quality features (i.e., RIN, pH, postmortem interval); see the *SI Appendix* for details]. *DRD1* clustered within a module including 2,452 probes. In this module, *DRD1* was not a hub gene (scaled within-module connectivity was close to the median). The module did not include any probes of the gene *DRD2*. The number of probes in this module was very high and difficult to study as a single unit. Recent evidence shows that further parceling of WGCNA modules can improve the detection of robust gene sets (48). To obtain the parceling, we used the hard-thresholding procedure described by Monaco et al. (49) based on the topological properties of the module (50, 51) (*SI Appendix*). Throughout the manuscript, we refer to this subset of coexpressed genes within the WGCNA *DRD1* module as the "*DRD1* gene set." Then, we investigated the biological functions of this gene set by using the software AMIGO2 (amigo2.geneontology.org/amigo) to assess the overrepresentation of gene ontology labels. Next, we sought to replicate in independent datasets the connectivity between members of the *DRD1* parceled gene set. With this aim, we used the publicly available dataset BrainEAC (www.braineac.org/) and the CommonMind Consortium (CMC) RNA sequencing data derived from postmortem human PFC (27) (Tables 1 and 2 and *SI Appendix*). We preprocessed BrainEAC and CMC expression values using the remove unwanted variation algorithm (RUV) designed to identify and subsequently remove latent confounding factors (52) (*SI Appendix*). We did not use explicit confounders. In Braincloud, we kept the original WGCNA for consistency with our prior work. Then, we used the permutation procedure described by Johnson et al. (53) (10,000 permutations) to test whether the gene–gene relationships between *DRD1* coexpression partners was significantly greater than a null distribution of random gene expression in these independent datasets. Finally, we asked whether gene expression covariation in both replication datasets had the same direction observed in Braincloud (*SI Appendix*).

DRD1 Polygenic Coexpression Index Computation. To assess whether gene-specific eQTLs predicted *DRD1* expression, we performed a local eQTL study by associating SNPs within 100 kbp up- and downstream *DRD1* with *DRD1* expression, as done in previous work (23). Then, we associated the GSE with SNPs to identify a pool of SNPs that, together, reliably predict coexpression. Hence, our aim was not to identify SNPs that, on their own right, predict coexpression—which requires statistics corrected for multiple comparisons. We selected SNPs falling into a window of 100 kbp up- and downstream the start and end position of each gene in the *DRD1* gene set (as in previous works) (21, 23) (see the *SI Appendix* for further selection criteria). To select SNPs included in the *DRD1*-PCI, we used the same criterion published by Pergola et al. (21): i.e., uncorrected $P < 0.005$ in the association with the GSE (*SI Appendix*). We also assessed the biological significance of the SNP set identified by interrogating the software Haploreg v. 4.1 (archive.broadinstitute.org/mammals/haploreg/haploreg.php) (28). Using Haploreg, we computed the statistics for overrepresentation of DLPFC (BA9/46) regulatory elements in our list of SNPs; note that also Braincloud data were obtained from BA46. Further analyses investigated the association of these SNPs with *DRD1* and other module genes (*SI Appendix*). Once we identified the SNPs associated with coexpression of the *DRD1*-related gene set, we generated the *DRD1*-PCI predicting the combined effect of such SNPs on *DRD1* GSE. This index actually refers to the entire gene set, and not just to *DRD1*; we indicate it by this name because we selected a priori the module including *DRD1* to investigate the transcriptomic context of this gene, which is key to WM performance and its underlying brain activity. The index computation is based on the signal detection theory and quantifies the magnitude of the differences between genotypic populations of each of the SNPs selected (*SI Appendix*). After validating the *DRD1*-PCI by means of cross-validation (*SI Appendix*), we replicated the association of the *DRD1*-PCI with the GSE using BrainEAC/CMC frontal cortex total gene expression data and genotypes. For these replications, we replaced genotypes with the weights detected in Braincloud. Based on the results in the Braincloud sample, we tested the negative correlations between the *DRD1*-PCI and replication GSEs using one-tailed *P* values (we were only interested in associations with the same direction in both the discovery and replication samples).

Assessment of WM Processing in Healthy Humans.

Participants. Three hundred and seventy-one unrelated adult Caucasian individuals participated in the study (Tables 1 and 2) (106 of them were also included in our recently published work on the *DRD2*-PCI) (21) and were genome-wide genotyped using previously published procedures (54). One hundred and fifty-two subjects formed the discovery sample for the fMRI and the behavioral study and performed the 1-, 2-, and 3-Back runs of the N-Back WM task (29). Furthermore, separate cohorts of 149 and 193 participants formed, respectively, the replication sample for the fMRI study (only 2-Back acquired) and the behavioral study (only 1- and 2-Back acquired). Inclusion criteria and sociodemographic assessments are detailed in *SI Appendix*. The present study was approved by the local IRB at the University of Bari Aldo Moro. Written informed consent was obtained from all participants after full explanation of all procedures, which were carried out according to the Declaration of Helsinki.

Neuropsychological task. We used the widely adopted N-back task to probe WM (14) in both the behavioral and the fMRI studies (*SI Appendix*). During fMRI, we used three runs of a block design version of the task: 1-Back vs. 0-Back; 2-Back vs. 0-Back, and 3-Back vs. 0-Back (named 01-Back, 02-Back, and 03-Back, respectively), each lasting 240 s. Participants in the fMRI replication sample performed the 02-Back run only. Participants included in the behavioral discovery sample performed three blocks of all of the three WM conditions, while those included in the behavioral replication sample performed 1-Back and 2-Back.

Imaging study. Blood oxygen level-dependent (BOLD) signal was recorded by a GE Signa 3T scanner (General Electric), using a gradient-echo planar imaging sequence to acquire 120 volumes for each N-Back run. We used Statistical Parametric Mapping 12 (SPM12) (www.fil.ion.ucl.ac.uk/spm) to analyze the fMRI data using standard procedures (55) (*SI Appendix*). Since we developed a continuous predictor of *DRD1* expression, we had the opportunity to control for nonlinear effects using the quadratic term of the *DRD1*-PCI as a covariate both in the behavioral and imaging analyses. In the fMRI discovery sample, we tested the association of the *DRD1*-PCI with brain activation using a general linear model (see the *SI Appendix* for model specification). We used F contrasts to investigate the association between *DRD1*-PCI and brain activity using both linear and quadratic terms of the *DRD1*-PCI. We masked results selecting the voxels in which the activity was higher during WM than the baseline to select areas belonging to the WM brain network. This brain network is the most plausible set of regions likely affected by the transcriptomic context of *DRD1* during this cognitive process. We generated the WM activity mask by identifying areas significantly activated separately per load (1-back versus 0-back, 2-back versus 0-back, and 3-back versus 0-back) and computed the conjunction null contrast between the three loads (whole-brain $P = 0.05$, uncorrected) (*SI Appendix*, Fig. S4). To correct the results of the linear and quadratic terms of the PCI for multiple comparisons, we used the stringent voxel-wise threshold of $P < 0.05$, whole-brain FWE-corrected, with a minimum extent of five voxels (~264 mm^3). To further detail our results, we displayed these clusters at $P < 0.001$, uncorrected, in the figures. We additionally explored the relationship between WM-related brain activity, WM performance, and *DRD1*-PCI, as well as the interaction with *DRD2*-PCI using mediation and moderation models (*SI Appendix*). In the replication sample, we computed an ANCOVA on the 2-Back > 0-Back contrast, using the same whole-brain FWE correction. Based on the results in the discovery sample, we computed only a *t* test on the negative linear term of the *DRD1*-PCI regression slope. Results are shown using the same criteria followed in the discovery sample and masked with the same WM network mask.

Behavioral analyses. We used differential WM accuracy [accuracy at the greater WM load minus accuracy at the lower WM load (Δ_{WM})] as a WM capacity measure (30, 31). This measure is associated with the increase of cognitive load in N-back sessions performed outside the scanner. It usually takes negative values and is a direct measure of WM capacity: i.e., greater values of differential accuracy indicate consistent WM performance in the face of load increase. In the behavioral discovery sample, we computed the differential accuracy between 2-Back and 1-Back (Δ_{2-1}) and between 3-Back and 2-Back (Δ_{3-2}) (31). Thus, we computed a repeated measures general linear model. For the behavioral replication sample, only Δ_{2-1} was available, and it was included in a general linear model (see the *SI Appendix* for model specification).

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