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ORIGINAL ARTICLE

Thalamic connectivity measured with fMRI is associated with a polygenic index predicting thalamo-prefrontal gene co-expression

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Abstract

The functional connectivity between thalamic medio-dorsal nucleus (MD) and cortical regions, especially the dorsolateral prefrontal cortex (DLPFC), is implicated in attentional processing and is anomalous in schizophrenia, a brain disease associated with polygenic risk and attentional deficits. However, the molecular and genetic underpinnings of thalamic connectivity anomalies are unclear. Given that gene co-expression across brain areas promotes synchronous interregional activity, our aim was to investigate whether coordinated expression of genes relevant to schizophrenia in MD and DLPFC may reflect thalamic connectivity anomalies in an attention-related network including the DLPFC. With this aim, we identified in datasets of post-mortem prefrontal mRNA expression from healthy controls a gene module with robust overrepresentation of genes with coordinated MD-DLPFC expression and enriched for schizophrenia genes according to the largest genome-wide association study to date. To link this gene cluster with imaging phenotypes, we computed a Polygenic Co-Expression Index (PCI) combining single-nucleotide polymorphisms predicting module co-expression. Finally, we investigated the association between PCI and thalamic functional connectivity during attention through fMRI Independent Component Analysis in 265 healthy participants. We found that PCI was positively associated with connectivity strength of a thalamic region overlapping with the MD within an attention brain circuit. These findings identify a novel association between schizophrenia-related genes and thalamic functional connectivity. Furthermore, they highlight the association between gene expression co-regulation and brain connectivity, such that genes with coordinated MD-DLPFC expression are associated with coordinated activity between the same brain regions. We suggest that gene co-expression is a plausible mechanism underlying biological phenotypes of schizophrenia.

Keywords Coordinated gene expression · Independent Component Analysis · Medio-dorsal nucleus · DLPFC · fMRI · Schizophrenia

Introduction

Schizophrenia is strongly associated with genetic risk, as consistently suggested by studies in twins, families (Bertolino and Blasi 2009) and in non-affected siblings of patients, which share with patients a series of neurobiological phenotypes (Gottesman and Shields 1971). In this regard, well-replicated brain phenotypes of schizophrenia include anomalies of the thalamus in terms of structure (Pergola et al. 2017b), activity (Smieskova et al. 2013) and connectivity (Giraldo-Chica and Woodward 2017). Previous studies have also suggested that the functional connectivity between this brain region and other areas of key relevance

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for schizophrenia such as the dorsolateral prefrontal cortex (DLPFC) is altered in this brain disorder (Antonucci et al. 2017), particularly during cognitive processing (Pergola et al. 2015). In this regard, we found anomalies in thalamo-prefrontal functional connectivity during attentional control in patients with schizophrenia and in their healthy siblings (Antonucci et al. 2016). More in detail, we found that during this cognitive process, as elicited with the Variable Attentional Control task (VAC, Blasi et al. 2005, 2010), the functional connectivity of a cluster overlapping with the medial dorsal thalamic nucleus (MD) is decreased in patients with schizophrenia and their healthy siblings within a network including the DLPFC (Antonucci et al. 2016). Taken together, this evidence suggests that anomalous thalamic functional connectivity is associated with familial risk for schizophrenia and is a biologically plausible candidate as an intermediate phenotype of schizophrenia.

Nevertheless, the genetic and molecular underpinnings of this anomaly remain unclear. In this regard, correlated gene expression across multiple brain regions has been reported to underlie brain connectivity patterns (Richiardi et al. 2015). Thus, it is possible that coordinated gene expression underlies synchronous interregional brain activity, and that this link between gene effects and brain physiology is relevant to schizophrenia. While this idea is suggestive, it is difficult to pinpoint which among the hundreds of genes and the diverse molecular mechanisms related to this brain disorder are implicated in specific neuroimaging phenotypes of brain connectivity (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). A possible approach to this issue lies on the clusterization of schizophrenia-related genes into biologically meaningful pathways (Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium 2015). Indeed, previous results on this topic revealed that some of the schizophrenia genes converge onto co-expression modules (Fromer et al. 2016). Thus, the investigation of patterns of gene co-expression between brain areas may add a crucial insight on the identification of mechanisms of modulation of interregional functional connectivity and of their anomalies in schizophrenia (Gaiteri et al. 2014).

The aim of this study is to investigate in healthy controls the relationship between: (1) thalamic functional connectivity within a network including the DLPFC, which we previously found associated with familial risk for schizophrenia (Antonucci et al. 2016); (2) a molecular interplay between the thalamus and the DLPFC (Gaiteri et al. 2014; Fromer et al. 2016), possibly relevant to biological pathways of this brain disorder. Specifically, we aimed to investigate whether coordinated MD-DLPFC gene expression related to schizophrenia reflects changes in thalamic functional connectivity during the VAC task, since attentional control is a cognitive process crucially linked to schizophrenia (Blasi et al. 2010). We performed this study in healthy controls to investigate the physiological basis of thalamic functional connectivity without the sources of bias that would confound data drawn from clinical populations. Figure 1 illustrates the study design. In more detail, we used a recently developed approach to associate post-mortem gene co-expression and schizophrenia-related phenotypes (Pergola et al. 2017a; Fazio et al. 2018). In particular, we analyzed the entire transcriptome to identify gene modules with coordinated expression between the MD and the DLPFC, and enriched for genetic risk loci of schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). To translate the knowledge gained from the post-mortem study into in vivo predictions in individuals engaged in the VAC task during fMRI, we identified genetic variants associated with the simultaneous expression of genes in the target modules. Then, we computed a polygenic index reflecting a proxy of simultaneous expression of module genes. Finally, we used this index as a regressor in an Independent Component Analysis (ICA) (Calhoun et al. 2009) on fMRI data collected in healthy controls while they performed the VAC task (Blasi et al. 2005, 2010). We used ICA because it allows the investigation of functional connectivity by separating a multivariate signal into temporally coherent functional networks (i.e., independent components, Calhoun et al. 2008). Therefore, this tool is considered reliable for disentangling networks subserving diverse and specific brain functions (Kim et al. 2009), allowing for a specific and dynamic characterization of the regional functional connectivity within these specific networks. Conversely, traditional seed-based connectivity methods only allow a general characterization of dynamics of brain functional connectivity.

We hypothesized that genetically indexed co-expression of the MD-DLPFC coordinated network would be associated with changes in the connectivity strength of thalamus within an attentional control-related brain network including DLPFC.

Materials and methods

Co-expression study

Identification of genes with coordinated MD-DLPFC expression

We used the publicly available post-mortem RNA sequencing dataset Brainspan (Allen Institute for Brain Science, Brainspan Atlas of the Developing Human Brain, http://brainspan.org/) (see Table 1A for demographic information) to investigate the coordinated expression between the MD and the DLPFC in healthy controls. To this aim, we computed for each available gene the within-subject Kendall's

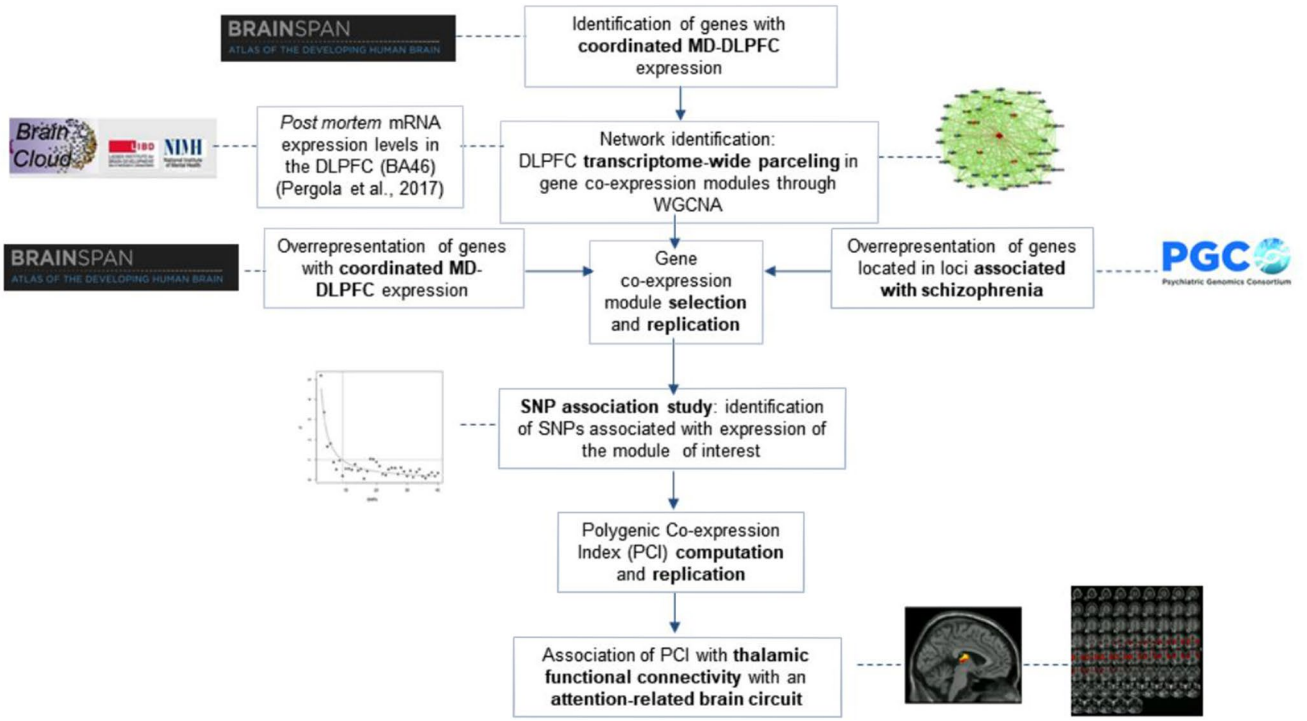

Fig. 1 Methods of the co-expression study. *DLPFC* dorsolateral prefrontal cortex, *BA* brodmann area, *WGCNA* weighted gene co-expression network analysis, *MD* medio-dorsal nucleus, *SNP* single-nucleotide polymorphism

Tau correlation index of gene expression between the two brain regions (Xu et al. 2010; Kumari et al. 2012). As done in a previous work (Richiardi et al. 2015), we obtained one p value of the correlation for each gene and Bonferroni-corrected alpha for multiple comparisons ($\alpha = 0.05/14{,}655$, where 14,655 were the number of tested genes). We selected gene modules based on Bonferroni-corrected p value ($\alpha = 0.05$). As a result, we obtained a list of 177 genes with coordinated MD-DLPFC expression (for a full description, see supplemental information Sect. 1 and Table S1).

Gene module selection based on the identified genes with coordinated MD-DLPFC expression

Network identification We used BrainCloud (Colantuoni et al. 2011) and Weighted Genes Co-Expression Network Analysis (WGCNA) to obtain a transcriptome-wide co-expression network in the post-mortem DLPFC of 199 healthy controls individuals. BrainCloud includes post-mortem mRNA expression levels of healthy subjects in Brodmann Area 46 obtained with oligonucleotide microarray paired with genome-wide genotypic information. Importantly, we did not perform a WGCNA analysis on the Brainspan dataset, in which we identified genes with MD-DLPFC coordinated expression (see "Identification of genes with coordinated MD-DLPFC expression"), because (1) its sample size ($N = 27$) would have been too small to perform a reliable WGCNA compared with the larger Braincloud dataset sample size ($N = 199$), and (2) the age range of the BrainCloud sample was closer to the age range of the fMRI sample. Instead, we used a network derived from a WGCNA analysis on the BrainCloud dataset reported previously (Pergola et al. 2017a), without identifying a new network; details are reported in the supplemental information (Sect. 2.1).

Module selection Differently from our previous work which prioritized modules based on candidate genes (Pergola et al. 2016, 2017a; Fazio et al. 2018), here we screened the entire network to investigate the genetic convergence between MD-DLPFC potential co-expression pathways and genetic risk for schizophrenia. With this aim, we used two criteria for module selection, i.e., the overrepresentation in the module of: (1) genes with coordinated expression between the MD and the DLPFC (see "Identification of genes with coordinated MD-DLPFC expression" and "Overrepresentation of genes with coordinated MD-DLPFC expression"); (2) genes located in the risk genetic loci for schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) (see "Overrepresentation of genes located in loci relevant to schizophrenia"). To further investigate the biological functions potentially subserved by the target co-expression modules, we computed gene ontology enrichment analysis using AmiGO2 online available tools (http://

Table 1 A. Demographic information of the samples of healthy controls included in the co-expression study. B. Demographic information of the sample included in the fMRI study

A

Sample name	Sample size	Female (male) (ratio)	Age mean ± SD (years)	Age range (years)	Ethnicity ratio (African Americans:Caucasians:Hispanics)
BrainCloud	199	60 (139) (0.43)	32 ± 20	0–78	105:94:0
Brainspan	27	12 (15) (0.8)	11 ± 14	0–40	10:15:2
BrainEAC PFC	123	33 (90) (0.37)	58 ± 18	16–102	0:123:0
BrainEAC Thalamus	124	33 (91) (0.36)	58 ± 19	16–102	0:124:0

B

Sample name	Sample size	Age (Mean ± SD)	Gender ratio (m:f)	Handedness (Mean ± SD)	WAIS IQ (Mean ± SD)	Socio-economic status (Mean ± SD)
fMRI study	265	27 ± 7	122:143	0.78 ± 0.36	109.15 ± 10.67	42.80 ± 15.79

All subjects included in the fMRI sample were genotyped for the SNPs selected for PCI computation. Age is expressed in years. Socio-economic status was assessed with the Hollingshead scale. Handedness was assessed with the Edinburgh Inventory

SD standard deviation, *PFC* prefrontal cortex

amigo.geneontology.org/amigo/landing, Gene Ontology database released 2017-06-09).

Overrepresentation of genes with coordinated MD-DLPFC expression: after having identified a gene set with coordinated gene expression between the MD and the DLPFC in Brainspan, we sought to determine which of these genes were co-expressed in the DLPFC in our Braincloud WGCNA. We used hypergeometric tests to estimate the overrepresentation of the MD-DLPFC gene set (see "Identification of genes with coordinated MD-DLPFC expression") in WGCNA modules (see "Network identification"). We selected modules that survived Bonferroni correction for multiple comparisons, where 67 were the number of modules tested, at $\alpha = 0.05$. We additionally used a permutation procedure to provide a more stringent enrichment test. At each run ($n = 10{,}000$), we permuted gene module assignments, generating 67 random modules, and performed the hypergeometric test for each random module. We then computed 67 p values and retained the lowest one at every run, thus generating a null distribution of p values (see supplemental information Sect. 2.2; empirical p value < 0.001). We focused on the gene module that was enriched both for MD-DLPFC coordinated expression and for schizophrenia-associated genes.

Furthermore, we evaluated the specificity of the overlap between the identified module and MD-DLPFC genes. We generated different gene lists with genes whose expression were correlated between the MD and 15 brain regions available in Brainspan (http://www.brainspan.org/static/home) and checked whether the overlap with the module of interest was still significant (empirical p value < 0.001, see supplemental information, Sect. 2.2, for a full description of the permutation procedure employed). Furthermore, we also checked whether the genes with coordinated MD-DLPFC expression were more expressed in the MD and in the DLPFC compared to random genes (see supplemental information, Sect. 2.2, for a detailed description of the procedure).

Overrepresentation of genes located in loci relevant to schizophrenia: Following the above-described module selection, we used hypergeometric tests to estimate, in the modules enriched for coordinated MD-DLPFC gene expression, the overrepresentation of the genes located in the 108 loci reported in the largest genome-wide association study on schizophrenia to date (11). As in previous works (Pergola et al. 2016, 2017a), we extended the loci by 100 kbp and obtained a list of 488 protein-coding genes. We corrected the p values of the hypergeometric tests with Bonferroni's rule ($\alpha = 0.05$) and also assessed significance through permutation test (threshold empirical p value as determined by Bonferroni correction, see "Network identification" and supplemental information Sect. 2.2.).

Module replication To assess the reproducibility of the co-expression of the modules selected based on the above-mentioned criteria, we used the publicly available post-mortem microarray dataset BrainEAC (http://www.braineac.org/) (Trabzuni et al. 2011), which contains mRNA expression data from the prefrontal cortex (PFC, $N=123$) and the thalamus ($N=124$) (Table 1A) of healthy controls. After preprocessing the data, we computed empirical p values of replication of gene–gene connectivity through a previously described permutation procedure (Johnson et al. 2015) (supplemental information, Sect. 2.3.).

SNP association study and Polygenic Co-Expression Index computation

To index the co-expression of the selected module, we computed a Polygenic Co-Expression Index (PCI) based on the effect of independent single-nucleotide polymorphisms (SNPs) on such co-expression. In particular, we first identified independent SNPs located within 100 kbp up- and downstream the genes within the module enriched for MD-DLPFC coordinated expression and schizophrenia risk. Subsequently, we performed a module-wide association study between these independent SNPs and the first principal component of the module (module eigengene—ME), through separate one-way ANOVAs, as reported in our previous work (Pergola et al. 2017a) (supplemental information, Sect. 3.). We did not correct the association statistics of each SNP for multiple comparisons, because our aim was not to identify markers able to predict co-expression on their own right. Instead, we aimed to define an ensemble of independent SNPs which afforded sufficient predictive power for module co-expression (Pergola et al. 2017a), and to this aim we used information theory.

In particular, we computed PCIs by assigning a weight to each genotype of each SNP based on the expression profile within different genotypic groups (Pergola et al. 2017a) (supplemental information, Sect. 3.). Higher values of the PCI reflect higher values of simultaneous expression of module genes, as measured by the ME. To select the SNP ensemble most reliably predicting module co-expression, we assessed the information content of each SNP using Shannon entropy, which is a measure of the amount of information (Shannon 1948). Specifically, we tested the entropy variation introduced by each individual SNP by computing 100 PCIs, each of them comprising an increasing number of SNPs (the first to the 100th SNPs ranked by association with the ME). Then, we used Cohen's d to detect the minimum number of independent SNPs included in the PCI, beyond which information increase was below a given threshold (Cohen's $d<1$; supplemental information, Sect. 3.). To investigate whether this selection was more informative than a random selection, we computed the entropy of PCIs composed by randomly selected SNPs, extracted from the same module. Finally, we compared the entropy distribution of our selected PCIs with those of the randomly calculated PCIs using Cohen's d. The whole analysis was performed with a bootstrapping procedure; specifically, 6000 bootstrap samples were used to compute entropy variations and to obtain effect size estimation.

Replication of the association between PCI and co-expression To assess the reproducibility of the association between PCI and co-expression of the selected module, we used pre-processed BrainEAC mRNA expression (see "Module replication") and BrainEAC SNP genotypes. We computed the first principal component of the module gene expression values separately in the PFC and in the thalamus, and assessed the variance explained by this index. We used genotypic weights derived from BrainCloud to compute the PCI. Then, we computed Pearson's correlations between the PCI and the first principal component of module gene expression in the two regions of interest. We used a one-tailed p value of $p<0.05$ as the cut-off for statistical significance of the results, because we were only interested in positive associations.

fMRI study

Sample determination and characteristics

To investigate association between PCI and thalamic functional connectivity in an attention-related network including DLPFC, we enrolled 265 healthy unrelated Caucasian adults from the region of Apulia for a functional magnetic resonance imaging (fMRI) experiment (Table 1B). Psychiatric diagnoses were excluded via the Structured Clinical Interview for DSM-IV (First et al. 1996). We obtained genome-wide genotypes for all participants (Rampino et al. 2017a, b). For each participant, we assessed handedness with the Edinburgh Inventory (Oldfield 1971), socio-economic status with the Hollingshead scale (Hollingshead and Redlich 1958) and intelligence quotient (IQ) using the Wechsler Adult Intelligence Scale—revised (Wechsler 1981). All subjects had no history of drug or alcohol abuse within the last 6 months, no neurological or psychiatric disorders or any other significant medical condition, no first-degree relatives with a diagnosis of psychiatric disorders, no history of head trauma with loss of consciousness, and no metal implants.

The experimental protocol was approved by the local ethics committee of the University of Bari Aldo Moro. All subjects were given a complete description of the study and its procedures. Written informed consent was obtained after a full understanding of the protocol according to the Declaration of Helsinki.

fMRI procedures and data analysis

All subjects performed the VAC task (Blasi et al. 2005, 2010; Antonucci et al. 2016), which allows to evaluate increasing levels of attentional control demand by asking subjects to indicate the direction of small, medium or big arrow stimuli, according to a cue word reported on screen (for a detailed description, see supplemental information Sect. 5.1). Data were acquired by a GE Signa 3T scanner (gradient-echo planar-imaging sequence, time repetition/time echo = 2000/30; 26 interleaved slices, thickness = 4 mm, gap = 1 mm; voxel size 3.75 × 3.75 × 5 mm; scans = 300; flip angle = 90°; field of view = 24 cm; and matrix = 64 × 64) and data analysis was performed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Images for each subject were realigned, unwarped and spatially normalized into a Montreal Neurological Institute template (for a detailed description of the preprocessing procedures, see supplemental information Sect. 5.2). Then, we performed one group spatial ICA on the whole dataset. We used the infomax algorithm (Bell and Sejnowski 1995) within GIFT v3.0a Matlab toolbox (http://icatb.sourceforge.net/) to decompose the multivariate VAC signal into its independent components. To do so, we used as GIFT inputs (1) the 300 normalized images of the VAC task, and (2) the vectors of onsets related to the three different and increasing level of attentional control required by the VAC task. ICA implementation and decomposition procedures (Antonucci et al. 2016) (see supplemental information Sect. 5.3) allowed to extract spatial independent components (ICs) from the entire dataset. Every spatial IC contains information about the simultaneous and synchronized activity between brain regions along the entire VAC task duration. Therefore, IC spatial maps reflect temporally coherent brain functional networks, as previously stated (McKeown and Sejnowski 1998; Calhoun et al. 2008, 2009).

To select the components of interest (COIs) for further investigations, we performed spatial correlations between each IC spatial map and (1) gray matter, (2) white matter, (3) cerebrospinal fluid templates, (4) the spatial map of an Attentional Control Template (ACN) described elsewhere (Blasi et al. 2005; Antonucci et al. 2016), and (5) the Attentional Control Component on which we previously found a main effect of familial risk for schizophrenia (Antonucci et al. 2016). Following a prior report, we considered for further investigation IC-template correlations with $R^2 > 0.01$ (Sambataro et al. 2010). Additionally, we filtered out ICs that did not include the thalamus and the DLPFC in their spatial maps by performing one-sample t-test on each COI spatial map ($p < 0.05$, whole brain Family-Wise Error—FWE corrected) and masking each one-sample t test with thalamic and DLPFC ROIs. Specifically, to define our thalamic ROI, we used the "Thalamus Atlas" (Krauth et al. 2010) to generate a mask of the bilateral thalamus composed by all thalamic nuclei included in the atlas; on the other hand, the DLPFC ROI was defined through the computation of a WFU Pickatlas mask (http://fmri.wfubmc.edu/software/pickatlas) including BA9, BA10 and BA46.

Thus, we investigated each significant COIs separately using a multiple regression analysis, with PCI as the continuous predictor, and age, gender, Hollingshead index, Edinburgh index, IQ scores and mean percentage of correct responses during the VAC task as nuisance covariates. We conducted one multiple regression analysis per COI. We masked these analyses using the bilateral thalamus as defined using the "Thalamus Atlas" (Krauth et al. 2010). We thresholded the results at $p < 0.05$, Family-Wise Error (FWE) corrected. To further control the results for multiple testing, we also applied a Bonferroni correction, for the number of investigated COIs ($n = 5$), to the p value of the cluster in which a main effect of PCI was present. COI loadings (expressed in arbitrary units) were then extracted from significant clusters using Marsbar (http://marsbar.sourceforge.net/).

Assessment of the specificity of the association between the Polygenic Co-Expression Index and thalamo-prefrontal functional connectivity

To further suggest that our findings are specifically related to coordinated MD-DLPFC gene expression, we investigated in the same COIs the potential effect of another PCI derived from the very same WGCNA (see "Identification of genes with coordinated MD-DLPFC expression"). In particular, we tested the previously published *DRD2*-PCI (Pergola et al. 2017a; Selvaggi et al. 2018), which indexed co-expression of a module including the D_2 dopamine receptor coding gene (*DRD2*). Furthermore, to assess whether the overrepresentation of schizophrenia genes yielded specific results compared to thalamo-prefrontal molecular pathways unrelated to schizophrenia risk, we additionally tested the PCI derived from another module of the network enriched for thalamo-prefrontal genes, but not for schizophrenia risk (i.e., *violet* module, see "Results", "Identification of genes with coordinated MD-DLPFC expression"). Based on the procedures described in "SNP association study and Polygenic Co-Expression Index computation", we found eight most relevant independent SNPs associated with *violet* module eigengene. However, since one SNP (rs2380737; Gene: C4orf19) was not available for our sample, we computed a 7-SNP *violet*-PCI, based on the remaining seven most relevant SNPs (see Supplementary Information, Table S1). SPM analyses were performed exactly as described above. Notably, all PCI tested in this work were derived from the very same WGCNA (Pergola et al. 2017a, b).

Anatomical parceling

Because of the complex architecture of the thalamus, we performed a descriptive anatomical parceling, as in our previous work (Antonucci et al. 2016). In particular, we used Marsbar to calculate the percentage of overlap between the significant cluster identified with ICA and thalamic nuclei, as identified with the "Thalamus Atlas" (Krauth et al. 2010). In this atlas, the borders of adjacent nuclei overlap. Therefore, we intersected adjacent masks of thalamic nuclei to obtain separate ROIs representing the borders of these nuclei (Antonucci et al. 2016). Then, we also reported the overlap between the significant cluster and the borders.

Results

Co-expression study

Module enrichment

The enrichment analysis revealed an overrepresentation of MD-DLPFC coordinated gene expression in two modules (*violet* and *cyan* modules) (Table S2), also surviving permutation test (empirical p value < 0.001). However, only one of these modules (*cyan*) was enriched both for coordinated MD-DLPFC gene expression (hypergeometric test, Bonferroni-corrected $p = 6.16 \times 10^{-5}$) and for schizophrenia-associated loci (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) (hypergeometric test, Bonferroni-corrected $p = 0.04$, Figure S2, Table S3 and supplemental information Sect. 6.). Permutation-derived empirical tests supported the significance of this finding ($p = 0.025$). In particular, *cyan* included 15 genes of the MD-DLPFC gene list and 15 genes of the PGC2 gene list out of 312 module genes. A single gene, *SRR*, was in common between the two lists. The first principal component of *cyan* (ME_{cyan}) explained 37% of the expression variance of the entire module. 276 out of 312 module genes were positively correlated with ME_{cyan} (mean Pearson r for these 276 genes = 0.614, standard deviation = 0.134). Furthermore, *cyan* was not significantly enriched for genes with coordinated expression between the MD and the other brain regions available in Brainspan (empirical p value ≤ 0.001), except for four cortical areas including the DLPFC (see supplemental information Sect. 6, supplemental Figure S3). This finding supports the specificity of the overlap between *cyan* co-expression and MD-DLPFC coordinated expression. Of note, the expression of MD-DLPFC genes was greater than the expression of a random gene set both in the DLPFC and in the MD (Figure S4—see supplemental information Sect. 6.), suggesting a functional role of these genes in both brain regions.

Module replication

We sought replication of the topological relationship between *cyan* genes found with BrainCloud both in the thalamus and in the PFC using BrainEAC. In particular, the latter database contained 297 genes included in *cyan* out of 312 present in BrainCloud. Results indicated that these genes were more connected than chance in the PFC (empirical replication p value < 0.001) and in the thalamus (empirical replication $p < 0.001$) (Fig. 2a, b). Thus, the gene–gene relationships were preserved in both brain regions.

The gene ontology enrichment analysis revealed that *cyan* was enriched for the ontologies reported in Table 2A (all $p < 0.05$, corrected), most notably "developmental process" (corrected p value = 0.03).

SNP association study and PCI computation

Based on the analysis of the entropy variation introduced by each individual SNP, we found ten most relevant independent SNPs, which were, therefore, used to compute the PCI (Table 2B, Fig. 2c). By definition, the PCI was positively correlated with the first principal component of *cyan* and explained a large portion of its variance (47%). To test whether this SNP selection was more informative than the one made by chance, we compared the entropy distribution of PCIs composed by randomly selected markers and our ranked-based PCIs. We found that these distributions were different, such that for every SNP selection, random PCIs had a different amount of information than our ranked-based PCIs (all Cohen's $d > 10$, Fig. 2d), therefore, suggesting that the information content of our selected SNP was different from chance.

Polygenic Co-expression Index replication

In the PFC replication dataset, the first principal component of the 297 selected genes explained 8.2% of the expression matrix variance. However, the association between this component and the PCI fell short of statistical significance ($r = 0.1$, $R^2 = 0.011$, one-tailed p value = 0.13). In the thalamus, the first principal component explained 7.4% of the variance and was significantly associated with the PCI ($r = 0.19$, $R^2 = 0.035$, one-tailed p value = 0.018).

fMRI study

ICA results

We extracted 45 independent components and 5 resulted spatially correlated with the spatial maps described in the methods. T tests revealed that all the 5 COIs included both thalamus and DLPFC ($p < 0.05$, FWE corrected). Regression

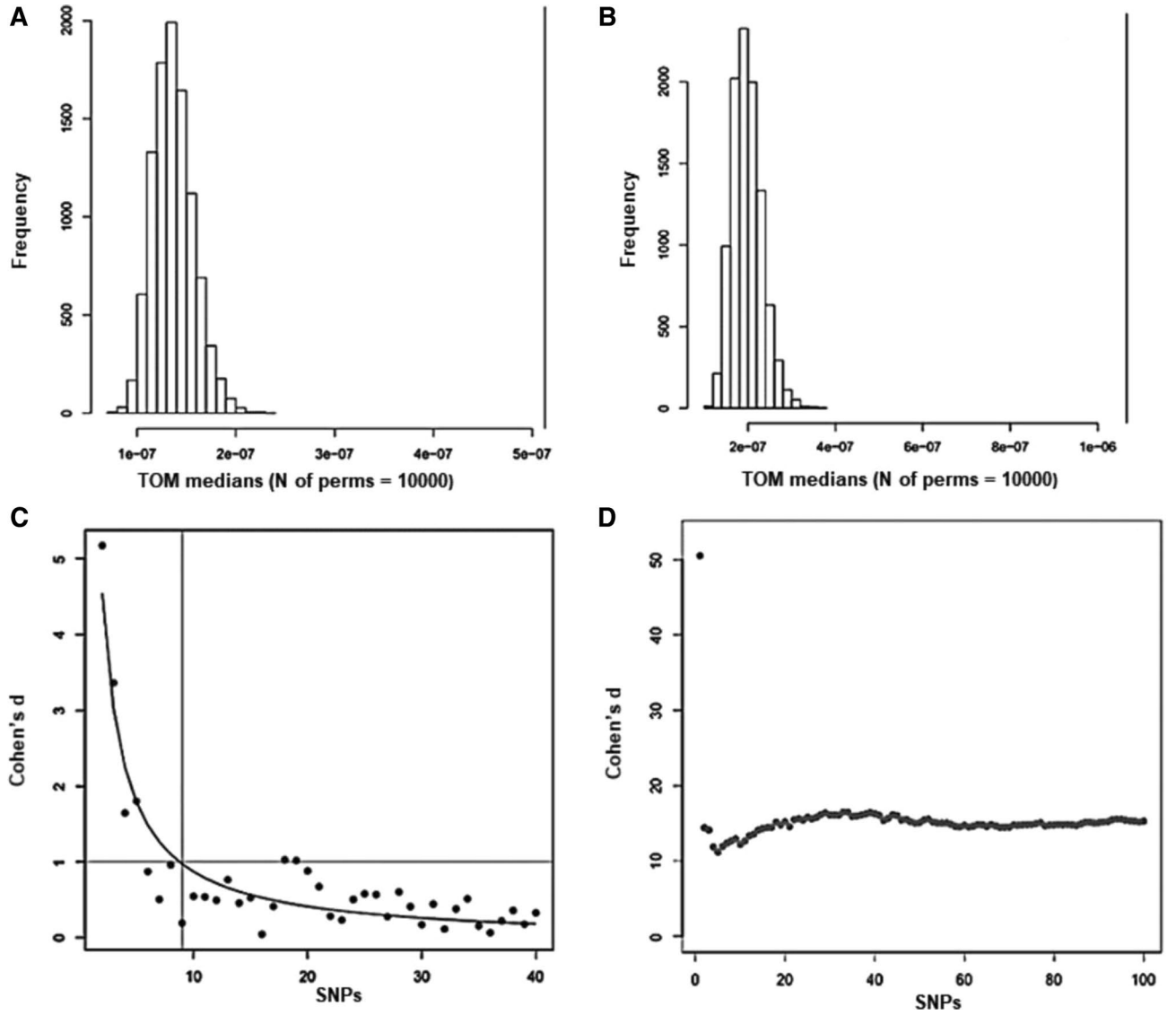

Fig. 2 Histograms showing the null distribution of the medians of 10,000 random co-expression modules Topological Overlap Matrices (TOMs) compared with *cyan* TOM median (vertical black line) in BrainEAC, **a** using data extracted from prefrontal cortex and **b** using data extracted from thalamus (see supplemental information). The topological relationships between *cyan* genes found with BrainCloud were preserved in both prefrontal cortex (**a**) and thalamus (**b**) using BrainEAC database. **c** Graphic representation of the marginal additive contribution of each SNP included in the PCI, based on the entropy variation analysis revealing ten most relevant SNPs; **d** graphic representation of the different entropy distributions of random SNP-based PCIs and ranked SNP-based PCIs (*x* axis: number of SNPs included in the PCIs; *y*-axis: Cohen's *d* value). For every SNP selection, random SNP-based PCIs had a different amount of information than our ranked SNP-based PCIs (all Cohen's $d > 10$). *SNP* single-nucleotide polymorphism

analysis revealed that PCI was positively associated with thalamic connectivity strength (MNI coordinates: − 10, − 14, 6; $Z = 4.59$; $k = 7$) (Fig. 3a, b) within a COI encompassing DLPFC, parietal cortex, thalamus, insula and putamen (Iq stability index: 0.82; correlation with the ACN COI: $R^2 = 0.012$; correlation with the attentional control component of Antonucci et al. 2016: $R^2 = 0.018$; Fig. 3c). Anatomical parceling indicated that the MD presented the largest overlap with the cluster we identified (Fig. 3d).

Assessment of the specificity of the association between the Polygenic Co-Expression Index and thalamo-prefrontal functional connectivity

Further analyses aimed to investigate the potential association of the five COIs (1) with a previously published PCI indexing a *DRD2*-related co-expression module and (2) with the PCI indexing *violet* module (not enriched for schizophrenia risk), revealed no significant associations.

Table 2 A. Results of the ontology enrichment of the *cyan* gene set surviving Bonferroni correction for multiple comparisons ($\alpha = 0.05$). B. Characteristics of the SNPs selected for PCI computation

A

Gene ontology biological process	Corrected *p* value	Fold enrichment
Cation binding (GO:0043169)	0.0003	1.62
Metal ion binding (GO:0046872)	0.0004	1.63
Ion binding (GO:0043167)	0.0011	1.45
Multicellular organism development (GO:0007275)	0.0110	1.53
Anatomical structure development (GO:0048856)	0.0166	1.48
Single-multicellular organism process (GO:0044707)	0.0228	1.45
Developmental process (GO:0032502)	0.0300	1.44
System development (GO:0048731)	0.0317	1.55
Single-organism developmental process (GO:0044767)	0.0420	1.45
Cellular component organization (GO:0016043)	0.0442	1.45

B

Marker	Position	Within sample MAF	Module expression association *p* value	Variation	Gene	Module gene	PGC association *p* value
rs1510480	Chr2:60598306	0.49	1.24×10^{-6}	Intergenic	*BCL11A*	*BCL11A*	6.27×10^{-3}
rs12233727	Chr4:54207103	0.47	5.16×10^{-6}	Intergenic	*PDGFRA*	*PDGFRA*	0.01
rs4307299	Chr8:11019115	0.48	3.40×10^{-5}	Intronic	*XKR6*	*XKR6*	0.14
rs3766506	Chr1:147665250	0.21	2.37×10^{-4}	Intronic	*BCL9*	*BCL9*	0.77
rs999856	Chr10:14019697	0.16	4.90×10^{-4}	Intronic	*FRMD4A*	*FRMD4A*	0.28
rs12461941	Chr19:37493748	0.16	5.22×10^{-4}	Intergenic	*ZNF793*	*ZNF793*	0.39
rs7923028	Chr10:80595439	0.15	5.68×10^{-4}	Intronic	*SH2D4B*	*TSPAN14*	0.35
rs10224467	Chr7:130146975	0.44	5.97×10^{-4}	Intergenic	*KLHDC10*	*KLHDC10*	0.10
rs1873746	Chr8:112370834	0.37	6.28×10^{-4}	Intronic	*CSMD3*	*CSMD3*	0.67
rs2052014	Chr14:72021100	0.19	7.39×10^{-4}	Intronic	*RGS6*	*RGS6*	0.06

GO gene ontology, *MAF* minor allele frequency, *PGC* psychiatric genomics consortium

Discussion

In this study, we aimed at investigating whether correlated gene expression across key regions for the pathophysiology of schizophrenia underlies variations in brain connectivity features at the core of the disorder. Thus, we investigated whether a genetic index of co-expression of a gene module enriched for coordinated MD-DLPFC gene expression related to schizophrenia was associated with the strength of functional connections of the thalamus in an attention-related brain network including the DLPFC during attentional control—a cognitive process affected by schizophrenia.

We identified a novel co-expression pathway associated with genetic risk for the disorder and with thalamic functional connectivity strength at the gene expression and imaging levels. Interestingly, thalamic functional connectivity was not associated with indexes of co-expression of schizophrenia-unrelated gene modules, suggesting the specificity of the association between the PCI and thalamo-prefrontal functional connectivity. Thus, our study offers for the first time a molecular insight about the mechanisms regulating thalamic connectivity within an attention network that may be potentially implicated in the pathophysiology of schizophrenia.

Thalamo-cortical coordination of gene expression and genetic risk for schizophrenia

The results of our co-expression study suggest that system-level brain phenotypes involved in the pathophysiology of schizophrenia are associated with mechanisms of gene co-expression which may also be relevant for this brain disorder, as indicated by the overrepresentation of schizophrenia risk loci in our co-expression module. Furthermore, they suggest novel genetic players that may modulate patterns of coordinated gene expression between the thalamus and the prefrontal cortex possibly implicated in the pathophysiology of the illness. It is also important to note that the gene module we identified was enriched for neurodevelopmental processes,

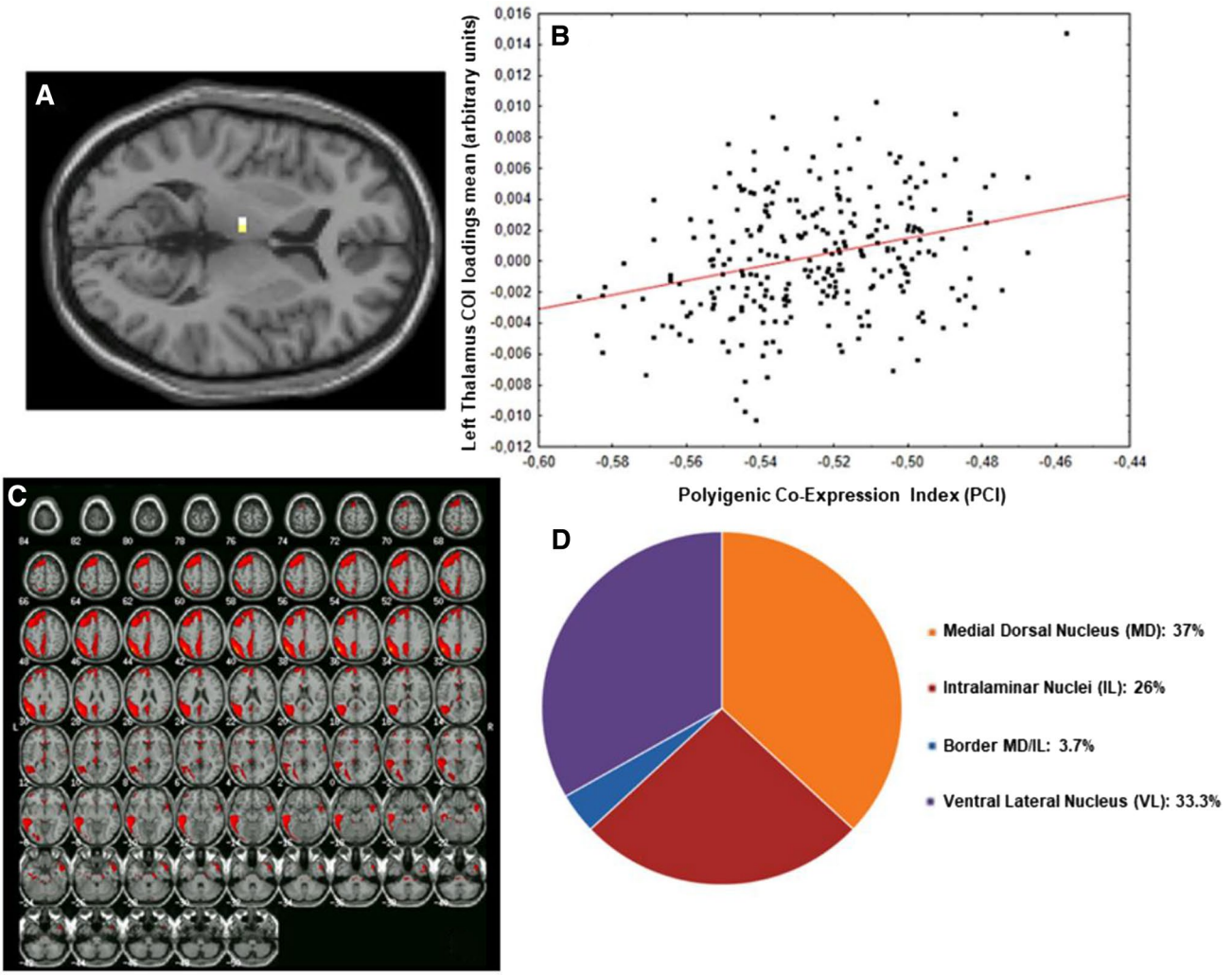

Fig. 3 **a** Axial image of the positive effect of PCI located in left thalamus. **b** Scatterplot of the positive effect of PCI on connectivity strength values extracted from the cluster depicted in **a**. **c** Spatial map of the Component of Interest (Iq = 0.82) in which a positive effect of Polygenic Co-Expression Index (PCI) was found. **d** Anatomical parceling of the cluster, located in the left thalamus, in which a significant effect of the PCI was found

consistently with current hypotheses on the pathophysiology of schizophrenia (Bertolino and Blasi 2009).

Interestingly, we found that the gene *SRR* was present both in the list of MD-DLPFC coordinated expression and in the list of schizophrenia genes. This gene is located in the region 17p13.3 and codes for serine racemase, which converts l-serine to d-serine (Wolosker et al. 1999), an endogenous ligand of the NMDA receptor (Matsui et al. 1995). The biological plausibility of this finding lies in the fact that both thalamo-cortical and cortico-thalamic connections are mediated by glutamate (Pakkenberg et al. 2009). Thus, our results should be interpreted in the framework of a relationship between glutamate, coordinated thalamo-prefrontal gene expression, and schizophrenia. Consistently, previous findings indicated decreased glutamate-related gene expression in thalamo-cortical neurons in schizophrenia (Sodhi et al. 2011). Furthermore, a SNP in SRR (rs4523957) has been associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) as well as with prefrontal brain activity and behavioral performance during attentional processes (Rampino et al. 2017a, b).

Several of the SNPs included in our PCI are located in genomic regions previously associated with phenotypes relevant to brain disorders or to the neurodevelopmental hypothesis of schizophrenia (Weinberger 1987). For example, rs1510480 is located in the *BCL11A* gene, which encodes a C2H2-type zinc-finger protein. BCL11A is a silencer of fetal hemoglobin expression (Sankaran et al. 2009). This gene has been associated with intellectual disabilities (de Leeuw et al. 2008) and with schizophrenia (Basak et al. 2015). Furthermore, this genomic region has been linked to

attention deficit hyperactivity disorder (Hinney et al. 2011), suggesting that this could be a trans-diagnostic locus of interest. This hypothesis is further corroborated by other findings indicating that *BCL11B*, which is closely related to BCL11A, has been previously associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) and is also part of the *cyan* gene module. Another SNP included in our PCI is located in the gene coding for CSMD3 (CUB and Sushi multiple domains 3), a protein expressed in fetal and adult brain and regulating neuronal migration, dendrite outgrowth, and synapse formation (Gunnersen et al. 2007). Importantly, variation in this gene has been associated with autism, bipolar disorder and schizophrenia (Magri et al. 2010; Malhotra et al. 2011). Taken together, our co-expression findings are consistent with previous reports and suggest relevance of genetic features previously associated with schizophrenia for mechanisms of thalamo-prefrontal connectivity.

Thalamo-cortical connectivity during attentional control as a possible intermediate phenotype of schizophrenia

Our fMRI results revealed a significant association between PCI scores and thalamic functional connectivity during attentional control, such that the lower the PCI (and thus the simultaneous expression of the module genes), the lower the functional connectivity of the thalamus within an independent component relevant to attentional control and including the DLPFC. Notably, anatomical parceling suggests that the thalamic cluster associated with the PCI overlaps with the MD. The MD is thought to be the most relevant thalamic nucleus modulating the activity of the prefrontal cortex (Pergola et al. 2012). The MD is also crucial for cognitive processing (Pergola et al. 2012; Schmitt et al. 2017). For example, the "cognitive dysmetria" model (Andreasen et al. 1998) proposed that cognitive deficits characterizing schizophrenia patients may rely on functional and structural abnormalities of thalamo-prefrontal network.

However, it is debated to what extent thalamic abnormalities are associated with state-related factors (Sim et al. 2006; Pergola et al. 2015). Indeed, several studies have reported functional thalamic anomalies also in non-affected siblings of patients with schizophrenia, thus supporting the notion that heritability may play a role in shaping such abnormalities in this brain disorder (Pergola et al. 2015; Antonucci et al. 2016). In this regard, a previous ICA study by our group (Antonucci et al. 2016) revealed that familial risk for schizophrenia is associated with decreased thalamo-prefrontal connectivity during attentional control. The present results highlight a gene co-expression pathway that may underlie the heritability of this brain phenotype and participate in its molecular underpinnings.

Taken together, our results support the hypothesis of a relationship between co-expression of genes involved in neurodevelopmental processes and thalamic functional connectivity during attention. Furthermore, they suggest a plausible molecular mechanism for thalamic connectivity anomalies at the network level in schizophrenia. Finally, they are also in line with the view that thalamic connectivity abnormalities are trait-dependent features of schizophrenia and that such traits may be fundamentally related with the control of gene expression in multiple brain regions.

Limitations

A limitation of this study is that we were unable to assess the thalamo-frontal gene correlations and co-expression networks in patients with schizophrenia. While the altered thalamo-cortical connectivity is a phenotype established by multiple studies including schizophrenia patients (reviewed by Pergola et al. 2018), we could not test any direct association between our post-mortem findings and this brain disorder. On the upside, our approach implicating the investigation of healthy controls allowed us to rule out biasing factors related to state variables of schizophrenia including pharmacological treatment, symptoms, or chronicity. In sum, our results may be informative on biological mechanisms possibly relevant to schizophrenia. However, their relevance for patients affected by this brain disorder should be addressed by further investigations.

Another limitation of this study arises from the type of data used for the WGCNA computation. Given that the Braincloud dataset only includes post-mortem DLPFC transcriptome-wide co-expression data, this may limit the interpretability of our findings. On the other hand, the Brainspan dataset was relatively small for performing a WGCNA (see "Network identification"). Therefore, future replication studies with larger dataset are warranted to investigate the generalizability of our gene expression results.

Furthermore, the association of the PCI with the module eigengene was not significant in the prefrontal replication dataset, although it was significant in the thalamus replication dataset. Possible explanations for this mixed evidence include: (1) the greater RNA integrity of BrainCloud compared to BrainEAC, (2) the evaluation of constitutive and alternative probes in BrainCloud, as opposed to using estimated total gene expression in BrainEAC, and (3) the fact that samples in the latter dataset were not specific for the DLPFC. Another limitation is represented by the thalamic parceling modalities: although we believe that our group-dependent parceling modality represents a reliable technique for further characterizing our fMRI results, we are aware that subject-specific parceling strategies might have increased the accuracy of the thalamic nuclei segmentation.

Conclusions

The uncertainty of schizophrenia-related clinical and brain phenotypes, and the complex genetic structure subtending its risk (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) make it extremely difficult to understand how gene effects implicate anomalous brain patterns relevant to schizophrenia. Our results suggest a connection between coordinated MD-DLPFC gene expression mechanisms and thalamic functional connectivity during attention, a brain phenotype of schizophrenia. Furthermore, they highlight that the investigation of co-expression patterns may be key for the identification of mechanisms of disease. Finally, they also suggest potential genetic loci of interest for schizophrenia and call for future research on the clinical translation of the present findings.

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Compliance with ethical standards

Conflict of interest Alessandro Bertolino is a stockholder of Hoffmann-La Roche Ltd. He has also received lecture fees from Otsuka, Jannsen, Lundbeck, and consultant fees from Biogen. Giulio Pergola has been the academic supervisor of a Hoffmann-La Roche collaboration Grant (years 2015–2016) that funds his and Antonio Rampino's salary. Antonio Rampino has received travel fees from Lundbeck. All other authors declare no biomedical financial interests and no potential conflicts of interest.

Ethical statement All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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