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Brain annotation toolbox: exploring the functional and genetic associations of neuroimaging results

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Abstract

Motivation: Advances in neuroimaging and sequencing techniques provide an unprecedented opportunity to map the function of brain regions and identify the roots of psychiatric diseases. However, the results from most neuroimaging studies, i.e. activated clusters/regions or functional connectivities between brain regions, frequently cannot be conveniently and systematically interpreted, rendering the biological meaning unclear.

Results: We describe a brain annotation toolbox that generates functional and genetic annotations for neuroimaging results. The voxel-level functional description from the Neurosynth database and gene expression profile from the Allen Human Brain Atlas are used to generate functional/genetic information for region-level neuroimaging results. The validity of the approach is demonstrated by showing that the functional and genetic annotations for specific brain regions are consistent with each other; and further the region by region functional similarity network and genetic similarity network are highly correlated for major brain atlases. One application of brain annotation toolbox is to help provide functional/genetic annotations for newly discovered regions with unknown functions, e.g. the 97 new regions identified in the Human Connectome Project. Importantly, this toolbox can help understand differences between psychiatric patients and controls, and this is demonstrated using schizophrenia and autism data, for which the functional and genetic annotations for the neuroimaging changes in patients are consistent with each other and help interpret the results.

Availability and implementation: BAT is implemented as a free and open-source MATLAB toolbox and is publicly available at http://123.56.224.61:1313/post/bat.
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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Advances in non-invasive neuroimaging techniques have allowed investigation of the neural basis of human behavior (Bennett *et al.*, 2016; Spiers and Maguire, 2007) and to search for the roots of psychiatric diseases (Abi-Dargham and Horga, 2016; Andreasen, 1988). Neuroimaging analysis generates results in clusters of voxels/brain regions or in functional connectivity (FC) links between pairs of voxels or brain areas with correlated activity. The biological interpretation of these results, however, remains difficult, and we often need to look up and summarize individual studies in the literature to find biological explanations. Since each study usually has a small sample size and the results may be under powered and have a high false discovery rate (Yarkoni, 2009; Yarkoni *et al.*, 2010), explanations based on these results may not be very reliable.

Recently, Neurosynth integrated results from tens of thousands of neuroimaging investigations, providing more reliable mappings between brain voxels and cognitive states than individual studies (Yarkoni *et al.*, 2011). Meanwhile, the Allen Human Brain Atlas (AHBA) was constructed and provided a comprehensive 'all genes-all structure' profile of the human brain (Shen *et al.*, 2012). These two datasets have provided us with comprehensive knowledge for understanding the human brain at multiple scales and with multiple types or modalities of investigation. However, a huge gap still exists in using these data to interpret neuroimaging results. The mappings between voxels to function in Neurosynth, and to gene expression profiles in the Allen Brain Atlas are fine-scale (voxel level) representations, which cannot directly provide functional or genetic meaning for brain regions consisting of clusters of voxels, or of the FCs between them. Therefore, for most neuroimaging analyses that generate results in the form of multiple brain regions or FCs, a rigorous statistical mapping from voxel-level representations (either functional or genetic) to region-level knowledge is needed.

In this research, we developed the brain annotation toolbox (BAT), which, when provided with voxel-level coordinates, transfers information from Neurosynth about which functions are associated with those coordinates, and from the AHBA about which genes are associated with those coordinates. BAT can perform functional and genetic annotation for many neuroimaging results, either in 3D-volume space or 2D-surface space, in the form of clusters/regions or FCs (see Fig.1 for details). One appealing application is that BAT can provide functional and genetic descriptors for different widely used brain atlases such as Brodmann (Brodmann, 1909), Automated Anatomical Labeling Atlas 2 (AAL2) (Rolls *et al.*, 2015) and Craddock 200 (Craddock *et al.*, 2012). And BAT can also help identify the potential genetic and functional characteristics of newly discovered regions, such as the 97 brain regions recently identified by the Human Connectome Project (HCP), whose functional roles and genetic properties remain unclear (Glasser *et al.*, 2016; Yeo and Eickhoff, 2016).

2 Materials and methods

2.1 Functional annotation analysis for given clusters/regions

The aim of the functional annotation analysis for clusters/regions was to provide a functional explanation or interpretation for given clusters/regions. The principle of our functional annotation analysis was the same as the widely used gene enrichment analysis, which assumes that the co-functioning genes for the abnormal biological process underlying the study are more likely to be selected as a relevant group by high-throughput screening techniques (Huang *et al.*, 2009a, b). Similarly, in neuroimaging research, voxels within a cluster/region have a higher probability to be co-activated by the same terms that are functionally related to the cluster/region, compared to voxels selected at random. For a given term in the Neurosynth database (details of the 217 Neurosynth terms we used and our selection criteria are provided in the Supplementary Method), the extent of activation of a given cluster/region was termed as the activation ratio (ACR). Supposing there are x_i activated voxels in the cluster/region i for the given term, the ACR_i is calculated as Equation (1).

$$ACR_i = \frac{x_i}{N_i} \quad (1)$$

where N_i is the number of voxels in the cluster/region i. It should be noted that all of our functional and genetic annotation analysis are at the group level.

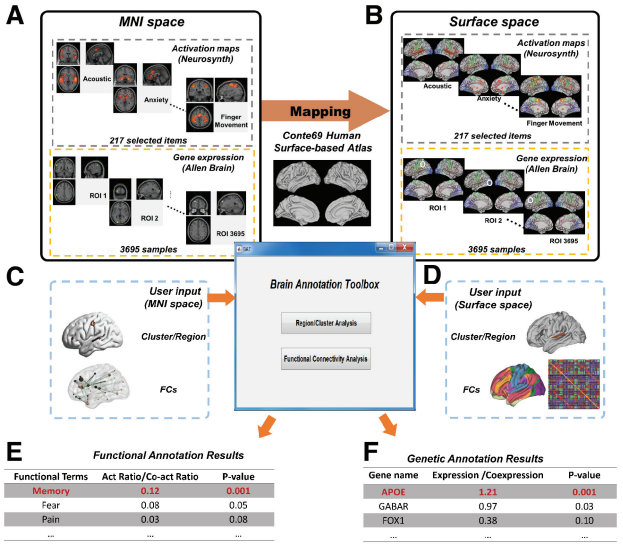

Fig. 1. Flow chart of functional and genetic annotation analysis. (**A**) Upper panel: the activation maps in MNI space for the 217 functional terms from the Neurosynth database. Bottom panel: 3695 AHBA samples with gene expression were employed and mapped to MNI space first. (**B**) Upper panel: the 217 activation maps in the MNI space were then mapped to the surface-based space by registering to the Conte69 Human surface-based Atlas (details are provided in the Supplementary Method). Bottom panel: the 3695 AHBA samples were mapping to the Conte69 Human surface-based Atlas as well. (**C, D**) Two general forms of neuroimaging analysis results, i.e. clusters/regions (C) and functional connectivities (D) (either in 3D MNI space or the 2D surface space) can be analyzed by the BAT. (**E**) BAT can perform functional annotation analysis for user-provided neuroimaging results and provide the most-related functional terms. (**F**) BAT can perform genetic annotation analysis for the user-provided neuroimaging results and identify the most correlated genetic correlates

Further, the statistical significance of the activation was evaluated by either Fisher's exact test or a non-parametric permutation approach (performed by randomly selecting voxels within the brain background mask. This background mask is specified by the user, which involves the brain regions to which the user wishes to compare the activated clusters/region). In the toolbox, functional annotation analysis can be performed for a cluster/region consisting of a single component with connected voxels (e.g. a single AAL2 region), a cluster/region consisting of multiple connected components (e.g. the activated clusters obtained from a specific task) and multiple clusters/regions (e.g. multiple AAL2 regions).

For a single cluster/region i, the above two kinds of statistical tests help users to infer which functional terms are significantly related to it. The Fisher's exact test is widely used in gene enrichment analysis (Huang *et al.* 2009a,b; Rivals *et al.*, 2007), and the null hypothesis is that there is no relation between whether a voxel lies within a cluster/region and whether the voxel is activated for a given term. Under this null hypothesis, we can model the number of voxels in a cluster/region that are activated by a given term by the Hypergeometric distribution. Supposing there are x activated voxels in the cluster/region i for the given term, we can get the P-value by simply computing the probability of observing x_i or more activated voxels in the cluster/region i, see Equation (2) for details.

$$p_i = 1 - \sum_{s=0}^{x_i-1} \frac{\binom{K}{s}\binom{M-K}{N_i-s}}{\binom{M}{N_i}} \tag{2}$$

where N_i and M are the number of voxels in cluster/region i and the background mask, respectively; and x_i and K are the number of activated voxels in the cluster/region and the background.

For the statistical test based on a non-parametric permutation test, three approaches are used, differentiated by the way in which the spatial structure of the voxel in the cluster/region is considered. The first one is the most efficient and is suitable for all forms of cluster/regions. It randomly selects non-overlapping voxels within the background (with the same number as those in the given clusters/regions) and regardless of their spatial relationship. The second is suitable for the clusters/regions consisting of a single spatially connected component. For example, to annotate a region in the AAL2 template, we select the same number of voxels as that in the given region and these voxels are also spatially adjacent in the background. The third is for the clusters/regions consisting of multiple spatially connected components. In this case, we randomly select non-overlapping connected components (with the same number as that in the given cluster/region), each consisting of spatially adjacent voxels (and with the same number as those in the components in the given cluster/region) from the background. After determining the voxel selection approach, BAT runs the permutation multiple times (the number of permutations can be defined by the user), to get a null distribution of the ACR for each term. The observed ACR is then compared with the null distribution to get the corresponding P-value.

2.2 Genetic analysis for the clusters/regions

Based on the gene expression data from the AHBA, the BAT's genetic analysis for the clusters/regions can provide the whole genomic gene expression profiles for the clusters/regions of interest and help to identify the differentially expressed genes (details of the gene expression dataset and its pre-processing procedures are provided in the Supplementary Method). For each AHBA tissue sample, we created a 6 mm sphere region of interest (ROI) centered on its Montreal Neurological Institute (MNI) centroid coordinate and terms these spheres as AHBA samples. The details for our genetic annotation analysis for clusters/regions are as follows.

First, with a given background mask, we retain AHBA samples with more than 50% of voxels that are also present in the background mask to perform further analysis (we term these samples as the background AHBA samples). Then, for each background AHBA sample, we map it to one of the given clusters/regions, that which has the largest number of overlapping voxels with this AHBA sample. The gene expression profile of each region/cluster is defined as the average gene expression of all the samples mapped to the cluster/region. We then adopt permutation analysis to identify the differentially expressed genes in the given clusters/regions (compared with samples in the background, the expression of the gene in the ROI is significantly increased/decreased). Two methods are used for sample selection in the background: (i) randomly selected AHBA samples from the background without repetition, and (ii) randomly selected AHBA samples in the background samples but not the ones that were already mapped to the region/ROI. Then for each cluster/region in each permutation run, we randomly select the same number of AHBA samples as those that are mapped to the cluster and calculate the average gene expression profiles across all selected samples. A null distribution for each gene was thereby obtained, allowing us to rank each gene in its null distribution and got its corresponding P-value for over-expression or down-expression.

2.3 Functional annotation analysis for FC

The BAT can also perform functional enrichment analysis for a FC or set of FCs constituting a network. A difference from previous analyses described for the BAT is that now the input data consist of a set of significant FC links. For example, we can determine the functions associated with the underlying FCs/networks identified by either a ROI-based approach or a brain-wide association study (BWAS). This is especially useful for the altered FCs identified in case-control studies.

An image map for the regions that are connected by the FCs and a list of all the FCs of interest are required to perform the analysis. First, to measure to what degree two regions connected by a FC are co-activated in a certain term, or task, we defined the co-activation ratio (CAR) of a FC for a term. For a FC l that connects regions i and j, its CAR for a specific functional term was calculated as Equation (3), as follows:

$$\mathrm{CAR}_l = \begin{cases} \dfrac{\mathrm{ACR}_i + \mathrm{ACR}_j}{2} & \text{if } \mathrm{ACR}_i \neq 0 \text{ and } \mathrm{ACR}_j \neq 0 \\ 0 & \text{if } \mathrm{ACR}_i = 0 \text{ or } \mathrm{ACR}_j = 0 \end{cases} \tag{3}$$

where ACR_i and ACR_j is the ACR for regions i and j, respectively.

For a functional network consisting of L FCs, its extent of activation for a specific functional term is defined as the mean co-activation ratio (MCAR), as defined in Equation (4).

$$\mathrm{MCAR} = \frac{\sum_{l=1}^{L} \mathrm{CAR}_l}{L} \tag{4}$$

where CAR_l is the CAR of the FC l which belongs to the network for the term.

In calculating what is described in this paper as 'functional connectivity', the activity of a node (i.e. a ROI such as an AAL2 area) for a particular search term was represented by the ACR of the node in that task. If in an analysis involving multiple FCs that some nodes appear n times, then the activity of that node is weighted by the number n so that its annotations contribute in this proportion to the annotations for this set of FCs.

Further, the significance of the network's MCAR is assessed using non-parametric permutation tests. Two methods for randomly selecting the regions connected by the FCs are used. The first is suitable for a brain network consisting of a moderate number of FCs (e.g. <20) and in which the brain regions connected by the FCs only occupy a small fraction of the brain (so that we can randomly select the same number of non-overlapping regions from the background). Using this method, in each permutation run, BAT randomly selects the same number of non-overlapping regions consisting of the same number of adjacent voxels as those in the resulting list from the background. The second method is suitable for FCs that connect regions from whole-brain atlases, e.g. the FCs obtained from regional-level brain-wide association analysis which produce a network with a large number of FCs that cover most of the brain. In such a situation, it is not feasible to randomly select the same number of non-overlapping regions from the background. We then randomly select the same number of regions as those in the FC list from the whole-brain atlas being used. Given the permutation method, the MACR of the FCs for each of the functional terms can be calculated based on the randomly selected regions. The null distribution of the MACR of the FCs for each of the functional terms is constructed after running the permutation multiple times. Based on the null distribution of a functional term, we can obtain a *P*-value for our observed MACR as the proportion of permutations in which with the randomly produced MACR is larger than the observed MACR.

2.4 Genetic analysis for the FCs

BAT can also identify genetic correlates for the given FCs, e.g. finding genes that might regulate the functional co-activation between two brain regions. First, the gene expression profile for each region involved in the given FCs is obtained (the same as for the 'Genetic analysis for the clusters/regions'). For each FC, the co-expression value (CEV) of a gene is defined as the outer product of its expression in these two regions (Hawrylycz *et al.*, 2015). As we used the normalized gene expression data (see Supplementary Material for details), if the gene expression in the two regions connected by the FC show high or low expression simultaneously, the gene will have a high positive CEV for the FC, whereas if they show opposite expression patterns, the gene will have a large negative CEV. For a functional network consisting of L FCs, we can use the mean co-expression value (MCEV) as defined in Equation (5) to represent the co-expression pattern of the gene for the function network.

$$\mathrm{MCEV} = \frac{\sum_{l=1}^{L} \mathrm{CEV}_l}{N} \tag{5}$$

where CEV_l is the CEV of the gene for the FC l which belong to the network.

Permutation analysis was applied to estimate the significance of the MCEV for each gene: first, in each permutation run, for each region in the FC list, we randomly select the same number of AHBA samples from the background as those mapped to the regions of the given FCs without repetition and calculate a new gene expression profile for the region, based on which we can obtain the MCEV for each gene. A *P*-value for the real MCEV was obtained for each gene.

3 Results

3.1 Functional and genetic annotation for well-known brain atlases

Using BAT, we performed functional and genetic annotation analysis for several widely known brain atlases, including the Brodmann (Brodmann, 1909), AAL2 (Rolls *et al.*, 2015), the new HCP atlas (Glasser *et al.*, 2016), Power 264 (Power *et al.*, 2011) and Craddock 200 (Craddock *et al.*, 2012), as detailed in Supplementary Table S3.

In particular, we highlight here the annotation results for Brodmann areas. We manually compared the functional annotation for 32 Brodmann areas (with significant annotation results, i.e. the region had at least one significant functional annotation by permutation test, $P < 0.05$) with those summarized in Wikipedia (wiki) (https://en.wikipedia.org/), to validate our approach. The annotations for all 32 regions provided by BAT were in agreement with those in wiki, i.e. there was a large extent of overlap between the functions we identified in these regions and those described in wiki, see Supplementary Table S4. The annotation results for other atlases can be found at our website (http://123.56.224.61/softwares). The functional and genetic annotations provided by BAT provide a valuable complement to these widely used atlases.

3.2 Functional and genetic annotation for the new brain atlas from HCP

In addition to traditional brain atlases, we also applied BAT to the recent HCP Brain Atlas (Glasser *et al.*, 2016). Using multi-modal data from the HCP, each hemisphere of the human cerebral cortex was parcellated into 180 different cortical areas. Among the 180 areas, 83 are consistent with previous reports, and 97 were newly identified in the HCP. This was an important advance, but did not address the genetic features underlying the 180 cortical areas, nor in detail the functions of each of the cortical areas (Yeo and Eickhoff, 2016).

To illustrate the information that BAT makes available for the 180 cortical areas in the HCP Brain Atlas, we describe the results for two selected areas: one is the hippocampus, and the other is a cortical area newly identified with the HCP Brain Atlas, the 'Middle Insular Area' (MI). As the functional and genetic annotations for the two regions are all available for the left hemisphere, here we focus on the left Hippocampus and MI, with details in Figure 2.

For the Hippocampus, 17 out of 217 functional terms, including 'memory', 'episodic memory', 'navigation', 'recall', 'learning task' etc. were found to be significantly associated with the hippocampus ($P < 0.05$, permutation test) (Fig. 2 and Supplementary Table S5). For genes, 4839 genes were found to be significantly over-expressed (i.e. genes expressed in this brain region or cluster or clusters more than in the rest of the brain) ($P < 0.05$, Bonferroni corrected). Gene enrichment analysis of these genes [using the software Toppgene (Chen *et al.*, 2009)] revealed that processes such as 'learning or memory' [$P = 1.31e{-}4$, Benjamini-Hochberg's False Discovery Rate procedure (BHFDR) corrected], 'learning' ($P = 5.10e{-}3$, BHFDR corrected) and 'memory' ($P = 7.83e{-}3$, BHFDR corrected) are significantly associated with these genes. The biological gene pathway 'long-term potentiation' underlying learning and memory was also found to be significantly enriched. These genes are also related to abnormal mouse phenotypes, such as 'abnormal synaptic transmission', 'abnormal long-term potentiation' and 'abnormal synaptic plasticity'.

Next, we summarize the results for a newly discovered cortical area, the MI, which is part of the insular cortex. BAT identified 105 out of 217 functional terms that were significantly related to activations produced in the MI area ($P < 0.05$, permutation test). Among the 105 functional terms, 12 could survive Bonferroni correction, including 'affective', 'awareness', 'reward', 'self', 'salience', 'pain', 'schizophrenia', 'somatosensory' and so on. For genes, we found that 415 genes were significantly over-expressed in the MI area

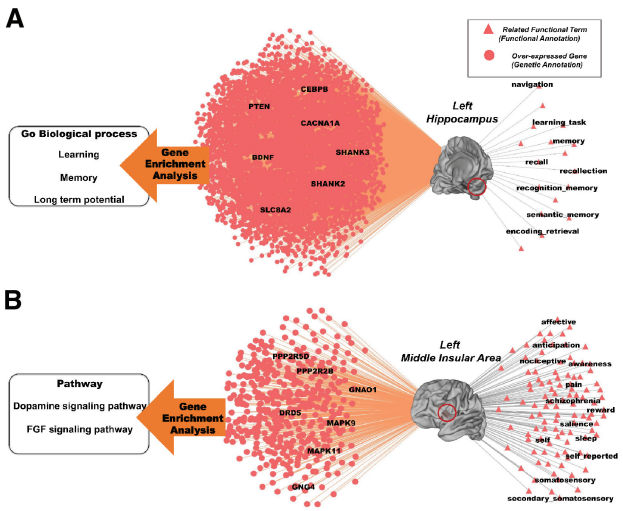

Fig. 2. Illustration of the functional and genetic annotations of two cortical areas in the HCP Brain Atlas. (**A**) Left Hippocampus: 17 functional terms, including memory-related ones such as 'memory', 'recognition memory' and 'Semantic memory', were found to be significantly associated with the left hippocampus. For genes, 4839 genes were found to be over-expressed including BDNF. Gene enrichment analysis shows that these genes are enriched in memory and learning related GO biological processes such as 'Learning', 'Memory' and 'Long term potentiation'. (**B**) Left MI area: 105 functional terms were found to be significantly related to the MI area, 'affective', 'awareness' 'reward', 'self', 'salience', 'pain' 'schizophrenia' and 'somatosensory' are among the 12 that can survive Bonferroni correction. A total of 415 genes were over-expressed in the MI area and enriched in the dopamine signaling pathway and fibroblast growth factor signaling pathway

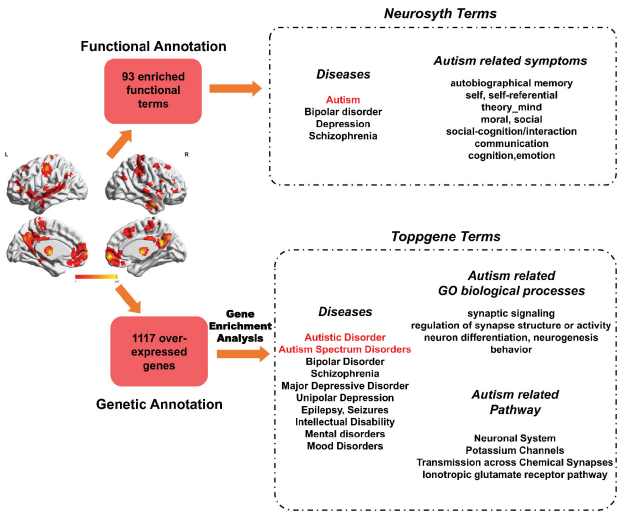

Fig. 3. The functional and genetic annotation for clusters obtained from the autism BWAS results. A total of 83 functional terms were found to be significantly related to the clusters, including 'Autism' and several autism-related symptoms such as 'autobiographical memory', 'communication', 'self-referential', 'theory of mind' and so on. Several Neurosynth terms for mental diseases, e.g. 'Bipolar disorder', 'Schizophrenia' and 'Depression' were also found to be significant. For genetic analysis, 1117 genes were identified to be over-expressed, which are also functionally enriched in the disease terms 'Autistic Disorder' and 'Autism Spectrum Disorders', and several autism-related GO biological processes and pathways. The gene enrichment analysis was performed using the Toppgene software

($P < 0.05$, Bonferroni corrected), significantly enriched in pathways that included the 'dopamine signaling pathway' ($P = 8.87e{-}3$, BHFDR corrected) and 'FGF signaling pathway' ($P = 1.30e{-}2$, BHFDR corrected). Interestingly, almost all the functional terms identified above were related to the dopamine pathway, the same as in the genetic annotation, suggesting consistency between the functional and genetic annotation, and thus verifying the usefulness of our approach. Detailed results for these two regions are provided in Supplementary Table S5.

3.3 Functional and genetic annotations for abnormal clusters identified in autism

To illustrate how BAT can help to gain insight into the biological meaning of neuroimaging results, we performed a functional and genetic annotation analysis for the clusters obtained in a BWAS of FC for autism (Cheng *et al.*, 2015), in which a statistical map is obtained by meta-analysis (with the Liptak–Stouffer Z-score approach) that integrates BWAS results from 16 imaging sites (418 patients and 509 controls). Then, Gaussian random field correction (cluster defining threshold: absolute $Z = 5.5$, cluster size $P < 0.05$) was performed and 23 clusters consisting of voxels that had significant FC changes were obtained.

We then fed these clusters to BAT, and found they are functionally enriched in 'autism' and autism-related functional terms including 'communication', 'self', 'social', 'theory of mind', etc. For genetic analysis, 1117 genes were found to be significantly over-expressed in the above clusters ($P < 0.05$, Bonferroni corrected), which were also significantly enriched in 'Autism Spectrum Disorders' ($P = 6.26e{-}04$, BHFDR corrected) and biological processes closely related to autism, such as 'synaptic signaling' ($P = 6.63e{-}16$, BHFDR corrected) (Zoghbi and Bear, 2012), 'neurogenesis' ($P = 3.40e{-}11$, BHFDR corrected) (Wegiel *et al.*, 2010), etc. Interestingly, these clusters were functionally and genetically enriched in several other psychiatric diseases such as schizophrenia and depression, indicating common genetic factors underlying these mental disorders (Smoller *et al.*, 2013), detailed in Supplementary Table S7. All the above functional and genetic annotation results are summarized in Figure 3.

3.4 Functional and genetic annotations for altered functional connectivities and networks in schizophrenia

To illustrate BAT's capability in helping to analyze neuroimaging results in the form of FC (or a brain network defined by a set of FCs), we further used BAT to perform functional and genetic analysis on the significantly changed FCs identified in chronic schizophrenia patients (Li *et al.*, 2017). A resting-state brain-wide association analysis was performed on multiple sites (with a total of 789 participants including 360 patients) (Li *et al.*, 2017), and the results were integrated by meta-analysis. We performed BAT on the 89 FCs that were significantly increased in chronic schizophrenia compared to controls.

We found that this dysregulated network of 89 FCs is significantly enriched in 43 functional terms (permutation test, $P < 0.05$), including 'schizophrenia' ($P = 0.0349$). Interestingly, these significantly increased FCs were also found to be significantly correlated with hallucination ($P = 0.0081$), which is an item in the Positive subscale of the Positive and Negative Syndrome Scale score (Li *et al.*, 2017). In addition, several other terms related to cognitive processes were also found to be significantly enriched, including 'attention' and 'memory', detailed in Supplementary Table S8. These cognitive functions are known to be impaired in patients with schizophrenia (Aleman *et al.*, 1999; Carter *et al.*, 2010). Finally, of all the identified functional terms, 'sleep' was the most significant

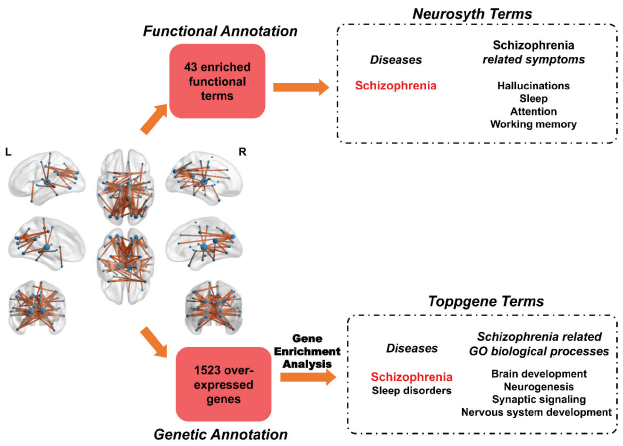

Fig. 4. Functional and genetic annotation results for the significantly increased FCs identified from chronic schizophrenia. The 89 increased FCs are significantly enriched in 43 functional terms including 'schizophrenia' and 'hallucination', 'attention' and 'memory'. A total of 1523 genes were identified to be significantly co-expressed in the regions connected by these FCs. These genes were significantly enriched in biological terms such as 'brain development' and 'neurogenesis'

($P < 1e{-}4$). Disturbed sleep is frequently encountered in patients with schizophrenia and is an important part of its pathophysiology (Cohrs, 2008).

For the genetic analysis, we selected those FCs whose associated brain regions had more than 5 AHBA samples, and this left 47 of the 89 FCs for genetic analysis. In total, 1523 genes were identified to be significantly co-expressed ($P < 0.05$, Bonferroni corrected) in the regions connected by these 47 FCs. These genes were significantly enriched in biological terms such as 'brain development' ($P = 1.31e{-}5$, BHFDR corrected), and 'neurogenesis' ($P = 2.43e{-}5$, BHFDR corrected), which are known to underlie the pathology of schizophrenia. Importantly, these genes were significantly enriched in the disease term 'schizophrenia' ($P = 4.49e{-}3$, BHFDR corrected), and were enriched in the mouse phenotypes involving 'abnormal sleep behavior' ($P = 3.35e{-}2$, BHFDR corrected), 'sleep disorders' ($P = 2.26e{-}2$, BHFDR corrected), see Figure 4.

In summary, the functional and genetic terms identified from the dysregulated network were both cross-validated, and highly consistent with the current understanding of schizophrenia, providing further evidence for the validity of the approach described here.

4 Discussion

Advanced neuroimaging techniques such as functional magnetic resonance imaging have generated gigantic neuroimaging data crucial for understanding the neural basis of behavior and for exploring the pathology of psychiatric disease. However, the results obtained in neuroimaging analysis, usually in the forms of clusters of voxels/brain regions or functional connectivities/networks, often remain hard to explain. In this research, we presented a toolbox that can provide functional and genetic annotations for brain atlas or neuroimaging results in the form of activation maps or FC, which is expected to shed insights into the biological meaning underlying these results.

In the field of bioinformatics, such an annotation analysis, gene functional enrichment analysis has already been employed to systematically dissect large 'interesting' gene lists from the high-throughput studies, and furthermore identify the most relevant biological processes (Huang *et al.* 2009a,b), based on the large amount of biological knowledge accumulated in public databases, i.e. Gene Ontology (GO). During the past decades, hundreds of gene functional enrichment analysis tools have been developed and employed by tens of thousands of high-throughput studies, providing valuable insights into the underlying biological meaning of the gene analysis results.

In sharp contrast, in the neuroimaging field, large databases such as Neurosynth (Yarkoni *et al.*, 2011) and AHBA (Hawrylycz *et al.*, 2012), have only recently been developed to provide functional/genetic knowledge for the human brain at the voxel level. However, tools for 'enrichment analysis' of neuroimaging results are still lacking. Inspired by gene enrichment analysis, we developed the BAT toolbox, which employs brain voxel-level functional and genetic knowledge to help systemically explore the region-level neuroimaging results (i.e. clusters/regions, or FCs).

BAT provides a novel method to harness the data from the Neurosynth and AHBA to perform functional and genetic annotation analysis for clusters/regions and FCs results, respectively. A user-friendly MATLAB GUI and 3D visual interface are also provided for users' convenience. We present four examples (for clusters/regions and FCs) in the Section 3 to illustrate the reliability of our annotation approach and to illustrate how to use BAT to search for the underlying biological meaning of the real neuroimaging results. It is noted that 'Neurosynth' also employed AHBA to identify the molecules that may participate in specific psychological or cognitive processes ('Neurosynth-Gene': http://neurosynth.org/genes/) (Fox *et al.*, 2014). However, it differs significantly from our approach in the following aspects: (i) the goal of 'Neurosynth-Gene' is to map individual cognitive phenomena to molecular processes, while the goal of BAT is to provide functional and genetic annotations for extensive neuroimaging results not necessarily confined to cognitive processes, e.g. from case-control studies. (ii) BAT can provide functional and genetic annotations and corresponding *P*-values for neuroimaging results in the form of FC or networks generated by whole-brain network analysis, which is widely used in the neuroimaging communities. This is not provided by 'Neurosynth-Gene'.

In developing the functional and genetic annotation methods, we took into account factors that might affect the results. Firstly, in the genetic analysis, in order to confirm that the size of the ROI does not significantly affect our genetic annotation results, we use 3, 6 and 9 mm spheres to define the ABHA samples and performed the genetic annotation analysis for regions in the AAL2 atlas. We found that the gene expression profiles for a certain region obtained using different ROI sizes were all highly correlated (3–6 mm: 0.98 ± 0.02; 6–9 mm: 0.97 ± 0.03; 3–9 mm: 0.96 ± 0.04). In addition, the region–region genetic similarity network obtained using the regional expression profiles from different ROI size were almost identical, see Supplementary Figure S2. All these results confirm that our genetic annotation results based on the regional expression profiles are not significantly affected by the ROI size. Secondly, in order to confirm that the results using permutation methods are stable and further assess the reproducibility of our method itself, we run functional and genetic annotation analysis for the abnormal clusters identified in autism and altered functional network in schizophrenia 10 times using the same parameters as previously used. The Pearson correlation of *P*-values between each two of the 10 runs is highly correlated for functional and genetic annotation analysis (functional annotation: 0.9943 ± 0.0001 and 0.9921 ± 0.0002; genetic annotation: $0.9999 \pm 6.17e{-}10$ and $0.9999 \pm 2.19e{-}9$). These results indicate that our results are not significantly affected by different permutation runs.

One attractive function of BAT is to help explore the newly discovered regions identified by neuroimaging technology, with unknown functions and genetic basis. We use the new parcellation of the human cortex provided by HCP as an example (Glasser *et al.*, 2016). The 180 cortical areas in the parcellation are distinguished by multi-modal data including anatomical measurements, task-related functional magnetic resonance imaging of seven tasks and resting-state FC in a subject cohort of 210 healthy young adults. This parcellation for the human cortex is at the highest resolution to date, but neither the function nor the genetic characterization of the 180 regions, especially for the 97 newly discovery regions, are clearly known. BAT can partly solve the problem: it can provide a complementary functional and genetic interpretation for the parcellation, and researchers using the new brain parcellation in their studies can use BAT to help explore the biological meaning of their results.

We now explain why functional and genetic annotations contain similar items for a number of brain regions. Previous investigations have identified the similarity between the gene co-expression network and resting-state functional network across regions, suggesting that the functional brain network is underpinned by the gene co-expression network (Krienen *et al.*, 2016; Richiardi *et al.*, 2015). To further validate our functional and genetic annotation, we used regions selected from the Brodmann, HCP, AAL2 and Cradock atlases and computed similarity matrices between all pairs of regions for the genetic and for the functional annotations. We found that these two similarity matrices corresponded significantly, as described next. We compared the following two networks: region by region co-activation networks, and region by region gene co-expression networks, for a given brain atlas. The former was constructed by calculating the Pearson correlation coefficient between the ACRs (of all 217 search terms or tasks) for each pair of brain regions; and the latter was obtained by calculating the Pearson correlation between the gene expression profile for each pair of brain regions. We found that the functional and genetic similarity matrices were significantly correlated, and this was found for all the brain

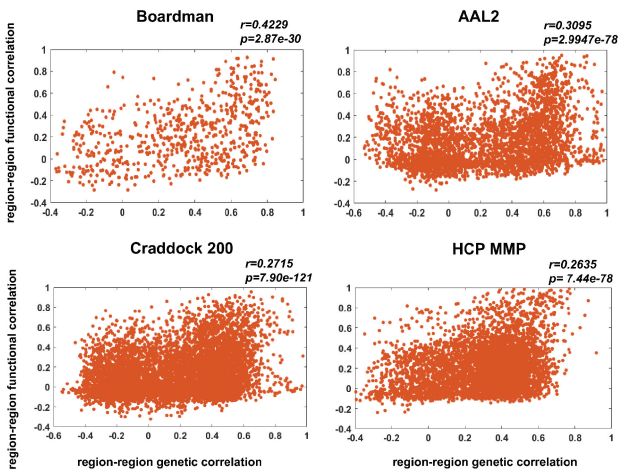

Fig. 5. A high correlation was found between the region by region co-activation network, and the region by region gene co-expression network for the Brodmann atlas, the AAL2 atlas, the Craddock atlas and the HCP atlas. Each dot in the figure represents an edge in the region by region network. The co-activation network was obtained by calculating the correlation coefficient between the ACRs (of all 217 terms or tasks) for each pair of brain regions in a given atlas, and the gene co-expression was obtained by calculating the correlation between the gene expression profile for each pair of brain regions in the same atlas

atlases (see Fig. 5; AAL2: $r = 0.310$, $P = 2.9947e{-}78$; BA $r = 0.423$, $P = 2.87e{-}30$; CRAD $r = 0.272$, $P = 7.90e{-}121$; HCP $r = 0.264$, $P = 7.44e{-}78$) adopted in this work, indicating that two brain regions with similar genetic expression profiles are more likely to have similar activation patterns.

BAT has a few limitations. First, selection bias caused by limitations in the data sources might introduced potential false positive results. The functional annotation analysis of BAT is based on the 217 selected functional terms for Neurosynth, which does not involve all the functional terms associated with all brain areas. For the genetic annotation analysis, the samples from the AHBA do not cover the whole-brain. Therefore, for regions/clusters or FCs that do not have enough AHBA samples (e.g. <5 samples) mapped to them, genetic analysis is not possible. Another important issue is the spatial dependence of the neuroimaging data. Although we provided a method to take this into account in the functional analysis, in our genetic analysis we could not do this as the AHBA samples were not evenly distributed across the whole-brain. Further efforts could involve integrating activation maps from all available meta-analysis databases [such as Brainmap (Fox and Lancaster, 2002)], reliable brain network parcellations obtained from large-scale neuroimaging datasets or meta-analysis [e.g. we provided the network-level annotation results for the altered FCs in schizophrenia patients (Section 3.4) based on Yeo's 7 and 17 networks (Yeo *et al.*, 2011) as an example, details in Supplementary Table S9] and gene expression profiles [such as that from Gene Expression Omnibus (Edgar *et al.*, 2002)], to avoid potential selection bias and provide a more comprehensive and reliable functional and genetic annotation for neuroimaging analysis. Secondly, it should be noted that we do not analyze the directed relationship between behavior-brain imaging-genetics in BAT, as the mediation analysis can only be performed using data at the individual level. However, our toolbox can provide candidate genes for further mediation analysis if behavior, neuroimaging and genetic data are available at the individual level. In addition to help users identify candidate genes, we also provide the whole genomic gene expression profiles associated with the ROI in the brain (such as clusters or regions linked by a FC), with which the users can perform further analysis, i.e. gene co-expression analysis. To sum up, the main aim of BAT is to provide a tool in the neuroimaging field, whose role is similar to that of gene enrichment tools in omics data analysis. It can provide potential functional and genetic correlates of the neuroimaging results and guide researchers in designing further experiments. Moreover, in BAT the significance levels can be set by users to levels that make the results reliable. An advantage of BAT is that the MATLAB source code is provided with the toolbox, allowing users to understand what is being computed, and to enable users to develop further enhancements.

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