LncRNA functional annotation with improved false discovery rate achieved by disease associations

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Abstract
The long non-coding RNAs (lncRNAs) play critical roles in various biological processes and are associated with many diseases. Functional annotation of lncRNAs in diseases attracts great attention in understanding their etiology. However, the traditional co-expression-based analysis usually produces a significant number of false positive function assignments. It is thus crucial to develop a new approach to obtain lower false discovery rate for functional annotation of lncRNAs. Here, a novel strategy termed DANet which combining disease associations with cis-regulatory network between lncRNAs and neighboring protein-coding genes was developed, and the performance of DANet was systematically compared with that of the traditional differential expression-based approach. Based on a gold standard analysis of the experimentally validated lncRNAs, the proposed strategy was found to perform better in identifying the experimentally validated lncRNAs compared with the other method. Moreover, the majority of biological pathways (40%–100%) identified by DANet were reported to be associated with the studied diseases. In sum, the DANet is expected to be used to identify the function of specific lncRNAs in a particular disease or multiple diseases.

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1. Introduction
Long non-coding RNA (lncRNA) is broadly defined as a type of non-coding RNA with a length of more than 200 nucleotides [1]. Tremendous evidences have shown that lncRNA can carry out diverse functions in biological processes [2] and is associated with many diseases [3], such as cancers [4], cardiovascular diseases [5], neurodegenerative diseases [6], metabolic diseases [7], and inflammatory diseases [8]. Currently, many computational methods for predicting lncRNA function have been developed [9], for instance, the differential expression analysis (DEA) combined with the weighted correlation network analysis (WGCNA) [10]. This method has been frequently employed for identifying co-regulatory relationships among lncRNAs and mRNAs in polycystic ovary syndrome [11] and discovering the cis-regulatory lncRNAs involved in vascular inflammation [12].

However, analysis based on co-expression usually results in a large number of false positive function assignments [9]. Currently, the lncRNA-disease association data supported by experiments are quite limited in the publications [13]. Specifically, only about 6,000 of over 90,000 lncRNAs have been characterized by experiments as “disease-associated” in human genome [14,15]. This may be attributed to the complex characteristics of lncRNA, including the higher expression variability across disease conditions [16–18], the susceptibility on expression/secondary structure to genetic variants [19–21], and the various levels of regulation on the coding genes (cis/trans) [2,18], etc.

So far, the analysis considering disease specificity into lncRNA functional annotation can improve the discovery of disease-associated lncRNA [16]. In particular, lncRNA-disease associations can be well-established via the single nucleotide polymorphisms (SNPs) type of genetic variants within lncRNAs [16] and condition-specific analysis estimated by the coefficient of variation
In this study, a novel strategy termed DAnet which combining cis-regulatory network was developed. The co-expression of the cis-regulatory IncRNAs and their neighboring protein-coding genes led to the discovery of functional IncRNAs in given disease [26]. It is therefore crucial to develop a new approach integrating diseased associations for obtaining lower false discovery rate (FDR) [17,22]. Moreover, lots of IncRNAs have been reported to regulate the expression of their neighboring genes (act in cis) [23–25].

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Table 1

| Type of Disease | Dataset ID     | No. of Sample in the specific dataset | Expression Unit (Experiment type) | No. of IncRNAs & mRNAs |
|-----------------|----------------|-------------------------------------|----------------------------------|------------------------|
| 8A20            | GSE13524       | 19 Alzheimer disease20 Healthy controls | TPM (RNA-Seq)                   | 12,937 IncRNAs & 18,969 mRNAs |
| 8A20            | GSE130740      | 12 Alzheimer disease10 Healthy controls | Normalized (RNA-Seq)             | 2,199 IncRNAs & 17,965 mRNAs |
| 8A20            | GSE125583      | 219 Alzheimer disease70 Healthy controls | nRPKM (RNA-Seq)                 | 2,803 IncRNAs & 18,852 mRNAs |
| 6A70            | GSE101521      | 30 MDD29 Healthy controls            | Normalized (RNA-Seq)             | 11,109 IncRNAs & 18,754 mRNAs |
| 6A70            | GSE102556      | 26 MDD22 Healthy controls            | RPKM (RNA-Seq)                   | 12,718 IncRNAs & 18,793 mRNAs |
| 6A20            | GSE112523      | 29 Schizophrenia28 Healthy controls  | RPKM (RNA-Seq)                   | 12,179 IncRNAs & 18,437 mRNAs |
| 6A41            | GSE65705       | 32 Myocardial infarction2 Healthy controls | RPKM (RNA-Seq)                 | 1,351 IncRNAs & 17,801 mRNAs |
| 6A41            | GSE127853      | 3 Myocardial infarction3 Healthy controls | RPKM (RNA-Seq)                 | 503 IncRNAs & 10,216 mRNAs |
| BD40            | GSE97210       | 3 Atherosclerosis3 Healthy controls  | Normalized signal intensity (Microarray) | 10,347 IncRNAs & 18,604 mRNAs |
| BD40            | GSE19025       | 4 Atherosclerosis unstable4 Atherosclerosis stable | RPKM (RNA-Seq)                 | 10,343 IncRNAs & 18,381 mRNAs |
| BC81            | GSE113013      | 5 AF-VHDV VHD                         | Normalized signal intensity (Microarray) | 10,347 IncRNAs & 18,604 mRNAs |
| BC81            | GSE108660      | 5 Atrial fibrillation5 Non-atrial fibrillation | Normalized signal intensity (Microarray) | 8,090 IncRNAs & 18,807 mRNAs |
| CA23            | GSE166388      | 15 Mild asthma4 Healthy controls      | Reads Count (RNA-Seq)            | 8,036 IncRNAs & 17,244 mRNAs |
| CA23            | GSE96783       | 21 Asthma30 Healthy controls          | Reads Count (RNA-Seq)            | 10,451 IncRNAs & 18,324 mRNAs |
| DD71            | GSE168686      | 14 Uterine cancer16 Healthy controls  | Reads Count (RNA-Seq)            | 1,756 IncRNAs & 17,355 mRNAs |
| 4A40            | GSE131525      | 3 SLE3 Healthy controls               | Reads Count (RNA-Seq)            | 6,031 IncRNAs & 16,972 mRNAs |
| 5A10            | GSE131526      | 12 Type-1 diabetes3 Healthy controls  | Reads Count (RNA-Seq)            | 6,798 IncRNAs & 16,458 mRNAs |
| 5B81            | GSE129398      | 12 Obesity10 Controls                 | Reads Count (RNA-Seq)            | 822 IncRNAs & 14,300 mRNAs |
| 5B81            | GSE1145412     | 8 Obesity8 Controls                   | TPM (RNA-Seq)                    | 6,896 IncRNAs & 16,595 mRNAs |
| 5A11            | GSE130099      | 6 Type-2 diabetes6 Lean controls      | Reads Count (RNA-Seq)            | 8,843 IncRNAs & 17,480 mRNAs |
| 2B93            | GSE141140      | 13 ALL4 Healthy controls              | Reads Count (RNA-Seq)            | 867 IncRNAs & 16,297 mRNAs |
| 2B91            | GSE144259      | 6 Colorectal cancer3 Healthy controls | RPKM (RNA-Seq)                  | 3,249 IncRNAs & 18,604 mRNAs |
| 2C6Z            | TCGA-BC [28]   | 115 Breast cancer113 Healthy controls | RPKM (RNA-Seq)                  | 14,097 IncRNAs & 19,631 mRNAs |
| 2D10            | TCGA_TC [28]   | 510 Thyroid cancer58 Healthy controls | Reads Count (RNA-Seq)            | 13,618 IncRNAs & 19,493 mRNAs |

In this study, a novel strategy termed DAnet which combining disease associations with cis-regulatory network was developed. In particular, disease-associated SNPs were first integrated for screening disease-associated IncRNAs. And then the CV of these IncRNAs was estimated to assess the condition-specific expression of IncRNAs in a specific disease. Moreover, the WGCNA-based co-expression network between IncRNAs and their neighboring protein-coding genes and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis were further conducted for identifying the function of the IncRNAs involved. Furthermore, experimentally verified IncRNA-disease associations were curated to evaluate the performance of this newly proposed strategy across 24 datasets involving eight types of disease based on classification of the ICD-11. Overall, the findings of this study can facilitate the discovery of disease-associated IncRNAs and their function in the specific disease.

2. Methods

2.1. Collection of the benchmark datasets for the analysis

For the function analysis of IncRNA in different type of diseases, a variety of microarray/RNA-seq data were collected by searching disease names in Gene Expression Omnibus (GEO) [27] and The Cancer Genome Atlas (TCGA) [28]. We considered several criteria: (1) the gene expression profiling was conducted using high throughput sequencing or IncRNA microarray for “Homo sapiens”, (2) the dataset consist of patient and control groups, (3) the raw data or normalized data were available, (4) the number of IncRNAs identified by disease-associated SNPs was more than zero, (5) the experimentally validated disease associated IncRNAs, which obtained from 5 public databases (LncRNAWiki [29], LncRNADisease [14], LncRNA2Target [30], Lnc2Cancer [31], and EVLncRNAs [32]), were available for the diseases and (6) multiple types of disease based on classification of the ICD-11. In total, 22 benchmark datasets were collected from GEO and two datasets were collected from TCGA, which included 16 diseases, divided into 8 types of disease according to the classification of ICD-11. Then, the IncRNA and mRNA expression matrices obtained from the 24 datasets of control-case studies were used for subsequent analysis. Table 1 demonstrates the disease type (ICD-11 code), dataset ID, the numbers of sample, the expression unit, and the number of IncRNAs and mRNAs for each dataset.

2.2. Collection of the SNP-disease association data for the identification of potential disease-associated IncRNAs

The SNP-disease association data were collected and used to identify potential disease-associated IncRNAs. First, we collected the 16 diseases associated SNPs and their locations from three well-known sources: GRASP2 [33], NHGRI-EBI GWAS Catalog [34], and GWASdb [35]. The significance level with p less than 5. × 10^{-8} is widely accepted in the genome-wide association studies [34]. Since many susceptible loci may only show moderate significance in association analysis, a p value of less than 1.0 × 10^{-3} was applied for collecting the disease-associated SNPs [35]. Then, we downloaded the chromosome information of IncRNAs from the GENCODE (v31, human reference genome hg38) [36] to map the disease-associated SNPs to the IncRNA region. In total, we collected 124,428 associations between 101,360 SNPs and the 16 diseases for further analyses, and 4,435 unique IncRNAs were found to be potentially associated with these diseases. Data details on the number of disease-associated SNPs and IncRNAs are shown in Supplementary Table S1. Finally, we exacted expression level of these IncRNAs in each dataset from raw IncRNA expression matrix, and
the number of the exacted lncRNAs based on disease-associated SNPs for each dataset is listed in Table 2.

### Table 2

| Disease Name          | Dataset ID   | No. of lncRNA in the specific dataset | No. of lncRNA based on disease-associated SNP | No. of experimental verified lncRNA | CV cutoff | CD cutoff |
|-----------------------|--------------|---------------------------------------|----------------------------------------------|-------------------------------------|-----------|-----------|
| Alzheimer disease      | GSE113524    | 12,937                                | 1680                                         | 5                                   | 400       | 400 kb    |
| Alzheimer disease      | GSE104704    | 2199                                  | 407                                          | 5                                   | 200       | 5 kb      |
| Alzheimer disease      | GSE123583    | 2803                                  | 537                                          | 5                                   | 400       | 50 kb     |
| Major depressive disorder | GSE101521  | 11,109                                | 1043                                         | 2                                   | 600       | 5 kb      |
| Major depressive disorder | GSE102556  | 12,718                                | 1098                                         | 2                                   | 1000      | 5 kb      |
| Schizophrenia          | GSE1112523   | 12,179                                | 917                                          | 3                                   | 300       | 5 kb      |
| Myocardial infarction  | GSE69705     | 1351                                  | 35                                           | 2                                   | 35        | 100 kb    |
| Myocardial infarction  | GSE127853    | 503                                   | 16                                           | 2                                   | 16        | NA        |
| Atherosclerosis        | GSE97210     | 10,347                                | 163                                          | 1                                   | 100       | NA        |
| Atherosclerosis        | GSE120521    | 10,343                                | 120                                          | 1                                   | 100       | 5 kb      |
| Atrial fibrillation    | GSE113013    | 10,347                                | 38                                           | 1                                   | 38        | NA        |
| Atrial fibrillation    | GSE108660    | 8090                                  | 33                                           | 1                                   | 33        | NA        |
| Asthma                 | GSE106388    | 8036                                  | 291                                          | 2                                   | 200       | 5 kb      |
| Asthma                 | GSE96783     | 10,451                                | 352                                          | 2                                   | 100       | 5 kb      |
| Lupus erythematosus    | GSE131525    | 6031                                  | 64                                           | 1                                   | 64        | 5 kb      |
| Ulcerative colitis     | GSE128682    | 1756                                  | 20                                           | 1                                   | 20        | 70 kb     |
| Type-1 diabetes mellitus | GSE115126  | 6798                                  | 283                                          | 3                                   | 200       | 5 kb      |
| Obesity                | GSE129398    | 822                                   | 46                                           | 1                                   | 46        | 5 kb      |
| Obesity                | GSE145412    | 6896                                  | 197                                          | 1                                   | 100       | 5 kb      |
| Type-2 diabetes mellitus | GSE133099  | 8843                                  | 1075                                         | 5                                   | 600       | 5 kb      |
| Acute lymphoblastic leukemia | GSE141140  | 867                                   | 12                                           | 1                                   | 12        | NA        |
| Colorectal cancer      | GSE144259    | 3249                                  | 43                                           | 6                                   | 43        | 300 kb    |
| Breast cancer          | TCGA_BC      | 14,097                                | 528                                          | 12                                  | 500       | 5 kb      |
| Thyroid cancer         | TCGA_T1      | 13,618                                | 8                                            | 1                                   | 8         | NA        |

2.3. Detection of the expression variability of lncRNA by condition-specific expression

The lncRNAs have higher expression variability pattern in diseases compared to normal conditions. LncRNAs with relative high expression variability pattern may indicate disease-related function while with relative low variability indicate function in normal condition [16,22]. The CV is the standard measurement for detecting the expression variability [16,22]. The CV is defined as “the ratio between the standard deviation of the lncRNA expression levels across the patients and its mean” [22]. In this study, we used this measurement to assess the variability of potential disease-associated lncRNAs. The CV value (ratio) was calculated for each lncRNA in disease samples, and the lncRNA with relative high CV value represents disease associated lncRNA. Finally, we ranked the CV values from high to low, and then identified the lncRNAs with top ranked CV values as the disease-associated ones. Meanwhile, different top numbers were used in the following optimization procedure. Among the top $K_{CV}$ (the top number of lncRNAs with the higher variabilities) lncRNAs across each dataset, the number of experimentally validated lncRNAs was computed ($N_{exp}$). When the number of lncRNA identified by SNPs ($N_{snp}$) was less than 100, the $K$ was equal to the $N_{snp}$. If else, the $K$ was from 100 to $N_{snp}$ with gradient of 100. When the $N_{exp}$ was maximum, the lower $K_{CV}$ was identified as the optimal value.

2.4. Construction of the cis-regulatory network based on lncRNAs’ neighboring genes

Co-expressed genes are more likely to be co-regulated and functionally associated, meaning that identification of the co-expressed neighboring protein-coding genes can be helpful in lncRNA function assignments [16,37,38]. Firstly, we collected the information of all 16,840 lncRNAs and 19,975 protein coding genes from GENCODE (V31, human reference genome hg38) [36]. After this, we obtained 10 candidate chromosome distances (CDs) based on the publications on genomic distance between the lncRNAs and their regulated neighboring genes. These CDs including: 5 kb [39], 10 kb [40], 20 kb [41], 50 kb [42], 70 kb [43], 100 kb [44], 200 kb [45], 300 kb [46], 400 kb [47], 500 kb [12]. Secondly, we calculated the neighboring genes within these CDs up/downstream of all lncRNAs based on the collected location information. Therefore, a collection of neighboring genes of identified disease-associated lncRNAs based on SNPs and optimal $K_{CV}$ was yielded. Thirdly, we constructed the co-expression network between identified disease-associated lncRNAs and their neighboring genes in different CDs for each dataset using WGCNA [10]. Moreover, optimization procedure was performed to determine the optimal CD across the benchmark datasets. Among the lncRNAs co-expressed with neighboring genes, the number of experimentally validated lncRNAs was computed ($N_{exp}$). When the $N_{exp}$ was maximum, the lower CD was regard as the optimal one. Finally, for the functional prediction, the co-expression network based on the optimal $K_{CV}$ and CD was constructed by WGCNA for each dataset. The network of selected module identified by WGCNA was illustrated by Cytoscape 3.7.2 (http://www.cytoscape.org/) [48] software.

2.5. Annotating the lncRNA function based on KEGG pathway

Groups of transcripts that are identified though clustering need to be subjected to a functional enrichment step to help in revealing the biological processes that these genes are involved in [16]. The KEGG pathway [49] is globally used for characterizing the function of disease-associated lncRNA. Herein, we performed the KEGG enrichment analyses by using the mRNAs that were found to be co-expressed with disease-associated lncRNAs. The statistical significance of KEGG pathway enrichments were determined with
the hypergeometric test. A p value less than 0.05 indicated a significant enrichment. Also, a chord diagram was constructed using R package “circlize” [50] to illustrate the enrichment results.

2.6. Evaluating the ability of DAnet on the function annotation of lncRNA

As a gold standard for verifying the DAnet analysis, 9,949 pairs of experimentally verified lncRNA-disease association were integrated from five databases including LncRNAWiki [29], LncRNADisease [14], LncRNA2Target [30], Lnc2Cancer [31], and EVLncRNAs [32], which provided many experimental verified lncRNAs for diseases. Two metrics were employed to evaluate the ability of the DAnet in characterizing the function of disease-associated lncRNAs. Both metrics were based on experimentally validated disease-associated lncRNAs. The metrics included: (1) percentage of successful prediction (Rate), and (2) enrichment factor (EF). The Rate (%) of DAnet and DEA (Supplementary Method S1) in characterizing the experimental verified lncRNAs was employed as the first metric to evaluate the performances. Also, EF was used to represent the comparison between the concentration of the experimentally verified lncRNAs in the identification results of DAnet/DEA and the concentration in the entire lncRNAs expression. The false discovery can be effectively evaluated by fully considering the experimentally validated disease associated lncRNAs [51]. The formula for EF is given:

\[
EF = \frac{N_{\text{true}}}{N_{\text{all}}} \frac{N_{\text{suc}}}{N_{\text{true}}} = \frac{N_{\text{suc}}}{N_{\text{all}}}
\]

where \(N_{\text{true}}\) denoted the number of experimental verified lncRNAs successfully characterized as ‘disease-associated’ by DAnet or DEA; \(N_{\text{suc}}\) represented the number of lncRNAs characterized as ‘disease-associated’ by DAnet or DEA; \(N_{\text{true}}\) was the number of experimental verified lncRNAs in the integrated experimentally verified lncRNAs-disease associations; and \(N_{\text{all}}\) indicated the total number of lncRNAs in the expression matrix. The EF no less than 1 indicated that there is an enrichment. The larger EF value represented the lower FDR [51].

3. Results

3.1. Identification of disease-specific lncRNA by SNPs across the benchmark datasets

More than 90% of disease-associated SNPs are actually located in the non-coding region (e.g., lncRNAs). The SNPs located in lncRNAs can either modify their secondary structure or affect their expression level [20]. As described in the Methods section, potential disease-associated lncRNAs of the 24 benchmark datasets were identified by disease-associated SNPs for DAnet analysis. The differential expressed lncRNAs were regarded as disease-associated lncRNAs for DEA (Supplementary Method S1) in characterizing the experimental verified lncRNAs was employed as the first metric to evaluate the performances. Also, EF was used to represent the comparison between the concentration of the experimentally verified lncRNAs in the identification results of DAnet/DEA and the concentration in the entire lncRNAs expression. The false discovery can be effectively evaluated by fully considering the experimentally validated disease associated lncRNAs [51].

As shown in Fig. 1, the Rate of DAnet was varied (from 2.6% for TCGA-TC to 100% for GSE113013 and GSE108660) and the Rate of DEA was also differed greatly (from 0% for 11 datasets to 33.3% for GSE125583). The EF no less than 1 indicated that there is an enrichment. The larger EF value represented the lower FDR [51].
for GSE106388). The Rate of DAnet was generally no less than DEA across 24 benchmark datasets. Moreover, among the 24 benchmark datasets, two datasets GSE97210 and GSE120521 from the atherosclerosis were collected from the microarray and RNA-Seq, respectively. We further compared the differences between the microarray and RNA-Seq data in terms of the originally detected lncRNAs, the potential disease-associated lncRNAs and the experimentally validated lncRNAs. As shown in the Supplementary Fig. S2, the total number of the originally detected lncRNAs for GSE97210 and GSE120521 was 10,347 and 10343, respectively. The number of lncRNAs detected by both GSE97210 and GSE120521 was 6836 (highlighted in blue and red lines). The number of potential disease-associated lncRNAs for GSE97210 and GSE120521 was 163 and 120, respectively. The number of shared lncRNAs was 111 (highlighted in green and red lines). In both GSE97210 and GSE120521, the experimentally validated lncRNA (CDKN2B-AS1) was identified via the DAnet. These findings indicate that both GSE97210 and GSE120521 are consistent in identifying the experimentally validated lncRNA.

3.2. Optimizing the KCV and CD parameters across the benchmark datasets

In order to identify more likely disease-associated lncRNAs, optimization procedure was performed to determine the optimal $K_{CV}$ and $CD$ across the benchmark datasets. As shown in Fig. 3, the optimal $K_{CV}$ represented in red square was varied across the datasets (from 8 for TCGA-TC to 1000 for GSE102556), and the CV of experimentally verified disease-associated lncRNAs was generally higher. Table 2 shows the optimal $K_{CV}$ value across the datasets. Moreover, as shown in Supplementary Fig. S3, the optimal CD represented in red square was different across the datasets (from 5 kb for 13 datasets to 400 kb for GSE113524). Table 2 shows the optimal CD across the datasets. For six datasets (GSE127853, GSE97210, GSE113013, GSE108660, GSE141140, TCGA_TC), the CD was not available.

3.3. The function of lncRNA in disease characterized by DAnet

3.3.1. KEGG enrichment analysis to characterize lncRNA function

Moreover, the co-expression network of lncRNAs and neighboring mRNAs was constructed under the optimal $K_{CV}$ and CD by WGCNA for each dataset. The network of module (contains the most genes with significant correlation) were displayed by Cytoscape. Four networks are shown in Fig. 4 A-D as examples, the light-yellow square represented the lncRNA and the blue dot represented the co-expressed mRNA in the cis-lncRNA regulatory networks, red edge represented the association between disease-associated lncRNA and neighboring mRNA. Other 14 networks are shown in Supplementary Fig. S4. For each dataset, the KEGG enrichment analysis was performed to characterize lncRNA function via the co-expressed mRNAs. A chord diagram was dawn for illustrating the significantly enriched pathways across different datasets (Fig. 4 E). As shown in Fig. 4 E, the enriched pathways reported to be associated with the disease studied were indicated in blue lines, and other pathways were shown in grey lines. The statistical results of disease-related pathways in each dataset are
shown in Fig. 4F. As shown, the percentage of disease-associated pathways were differed from 40% to 100% across datasets. The detailed descriptions on relevance between disease and pathways are provided in Supplementary Table S2.

3.3.2. Association between lncRNAs identified by DANet and the specific disease

Finally, the relationships of lncRNAs and diseases were systemic manually searched. As illustrated in Fig. 5, 41 directly disease-associated lncRNAs were identified for most diseases (blue lines). In particular, 13 lncRNAs were identified for Alzheimer disease (orange square, 8A20), three for major depressive disorder (brown square, 6A70), four for schizophrenia (brown square, 6A20), 12 for myocardial infarction (blue square, BA41), two for atherosclerosis (blue square, BD40), six for asthma (pink square, CA23), one for lupus erythematosus (purple square, 4A40), one for ulcerative colitis (turquoise square, DD71), five for obesity (yellow square, 5B81), six for type-2 diabetes mellitus (yellow square, 5A11), three for colorectal cancer (green square, 2B91), six for breast cancer (green square, 2C6Z). The detailed descriptions on relevance between lncRNAs and the specific disease are provided in Supplementary Table S3.

Meanwhile, as illustrated in Fig. 5, the lncRNAs (red dots) associated with multiple diseases were identified. Specifically, two lncRNAs (LINC-PINT, GAS5) were associated both with Alzheimer disease and type-2 diabetes mellitus [52–56], SOX2-OT was associated with Alzheimer disease and asthma [57,58], CCDC39 was associated with asthma and schizophrenia [59,60], HCP5 was associated with asthma and breast cancer [61,62], IFNG-AS1 was associated with asthma and ulcerative colitis [63,64], CDKN2B-AS1 was associated with five diseases including Alzheimer disease, myocardial infarction, atherosclerosis, type-2 diabetes mellitus, and breast cancer [65–70].

4. Discussion

Functional annotation of lncRNAs in diseases has attracted great attention for understanding disease etiology. In this study, we pro-
posed a novel strategy termed DAnet by combining disease associations with cis-regulated network between lncRNAs and neighboring protein-coding genes for improving the functional annotation of lncRNAs. The strategy mainly consists of three procedures including: (1) identifying potential disease-associated lncRNAs based on disease-associated SNPs, (2) detecting more likely disease-associated lncRNAs based on expression variability, (3) developing cis-regulated networks between disease-associated lncRNAs and their neighboring protein-coding genes. To widen the scope of DAnet to other RNA-seq or Microarray data, the code...
of DAnet was provided in Supplementary Method S2. DAnet can be expected to identify the specific lncRNA function in the given disease.

Primarily, based on the analysis of 24 datasets involving 16 diseases, the Rate value of DAnet was overall higher than the DEA, which indicates that the performance of DAnet could be better than traditional differential expression-based analysis on identification of experimentally validated lncRNA. In addition, the EF of DAnet was overall higher than the DEA. All EFs of DAnet were higher than 1. These findings indicate the superior capacity of DAnet in controlling the false characterization of lncRNA function. Furthermore, during the optimization procedure for determining the optimal $K_{CV}$, we found that the experimentally verified disease-associated lncRNAs were generally with higher CV values. This finding is consistent with those reported by other investigators [16–18]. Under the optimal $K_{CV}$, the optimal CD was not available for these six datasets (GSE127853, GSE97210, GSE113013, GSE108660, GSE141140, TCGA_TC). This may be attributed to the effect of the small number of samples and the few numbers of lncRNAs/mRNAs in the co-expression analysis [71]. Finally, the KEGG enrichment results indicate most biological pathways identified by DAnet were associated with the corresponding disease (from 40% to 100%). And by DAnet, directly diseases-associated lncRNAs were identified for most diseases. Moreover, lncRNAs associated with multiple diseases were also identified.

5. Conclusions

A new strategy integrating disease associations was developed for obtaining the lower false discovery rate in functional annotation of lncRNAs. The analysis of 24 datasets involving 16 diseases, indicated that the performance of DAnet could be better than traditional differential expression-based on identification of experimentally validated lncRNA, and the most biological pathways identified by DAnet were associated with the studied diseases. This provides a way to study the function of lncRNA in diseases from another aspect. In sum, DAnet is expected to identify the specific lncRNA function in the given disease.

Contributors

J.T. and Y.W. conceived the idea and supervised the work. Y.W., J.Z., and X.W., performed the research. Y.W., J.Z., X.W., Adu-Gyamfi E., L.Y., T.L., M.W., Y.D., and F.Z. prepared and analyzed the data. J.T. and Y.W. wrote manuscript. All authors reviewed and approved the final version of the manuscript.

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Yongheng Wang: Formal analysis, Writing – original draft, Writing – review & editing, Visualization. Jincheng Zhai: Formal
analysis. Investigation. Xianglu Wu: Investigation, Visualization. Enoch Appiah Adu-Gyamfi: Validation, Writing – review & editing. Lingping Yang: Investigation, Validation. Taihang Liu: Validation. Meijiao Wang: Validation. Yubin Ding: Project administration. Feng Zhu: Conceptualization, Project administration. Yingxiong Wang: Conceptualization, Supervision, Funding acquisition. Jing Tang: Conceptualization, Writing – original draft. Writing – review & editing. Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] Marchese P, Raimondi I, Huarte M. The multidimensional mechanisms of long noncoding RNA function. Genome Biol 2017;18(1):1-20. https://doi.org/10.1186/s13059-017-1278-0.
[2] Kopp F, Mendell JT. Functional Classification and Experimental Dissection of Long Noncoding RNAs. Cell 2018;172(3):393-407. https://doi.org/10.1016/j.cell.2018.01.011.
[3] Chen G, Wang Z, Wang D, Qiu C, Liu M, et al. LncRNADisease: a database for long-non-coding RNA-associated diseases. Nucleic Acids Res 2013;41(DDatabase issue):D983-986. DOI: 10.1093/nar/gks1099.
[4] Niknafs YS, Han S, Ma T, Speers C, Zhang C, Wilder-Romans K, et al. The lncRNA database, lncRNAdb: a comprehensive database for long non-coding RNAs. Nucleic Acids Res 2018;46(Database issue):D1193-D1198. https://doi.org/10.1093/nar/gky1029.
[5] Micheletti R, Plaisance I, Abraham BJ, Sarre A, Ting C-C, Alexanian M, et al. The long non-coding RNA lncRNA-Wipser controls breast cancer progression. Nat Commun 2016;7(1):1-7. https://doi.org/10.1038/ncomms11348.
[6] Millan MJ. Linking deregulation of non-coding RNA to the core pathophysiology of Alzheimer’s disease: An integrative review. Prog Neurobiol 2017;156:1-58. https://doi.org/10.1016/j.pneurobio.2017.03.004.
[7] Moran L, Akerman L, van de Bunt M, Xie R, Benazra M, et al. Human beta cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically expressed, and associated with diabetes phenotypes. Nucleic Acids Res. 2015;43(Database issue):D799-804. https://doi.org/10.1093/nar/gku1167.
[8] Cheng L, Wang P, Tian R, Wang S, Guo Q, et al. LncRNA-Target v2.0: a comprehensive database for long non-coding RNA-target interactions and clinical applications. Nucleic Acids Res 2019;47(Database issue):D135-D139. https://doi.org/10.1093/nar/gky1031.
[9] Ecker S, Pancaldi V, Rico D, Valencia A. Higher gene expression variability in noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. Nat Genet 2016;48(10):1142-50. https://doi.org/10.1038/ng.3677.
[10] Bao Z, Yang Z, Huang Z, Chen Y, Shan S, Soares F, et al. Risk SNP-Mediated Promoter-Enhancer Switching Drives Prostate Cancer through lncRNA PCA91. Cell 2018;174(3):564-575.e18.https://doi.org/10.1016/j.cell.2018.06.014.
[11] Guo H, Ahmed M, Zhang F, Yao CQ, Li SiDe, Liang Y, et al. Modulation of long noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. Nat Genet 2016;48(10):1142-50. https://doi.org/10.1038/ng.3677.
[12] Wang J, Wu Y, Wang J, et al. LncRNA-disease association prediction based on the Naive Bayesian classifier. Plos One 2019;14(8):e201529. https://doi.org/10.1371/journal.pone.0201529.
[13] Yu J, Xuan Z, Feng X, Zou Q, Wang L. A novel collaborative filtering model for the prediction of protein-protein interactions. Bioinformatics 2013;29(13):1665-71. https://doi.org/10.1093/bioinformatics/btt261.
[14] Bao Z, Yang Z, Huang Z, Zhou Y, Cui Q, et al. LncRNA-Disease: an updated database for human long non-coding RNA-associated diseases. Nucleic Acids Res 2019;47(D1):D1034-D1037. https://doi.org/10.1093/nkx905.
and single-molecule resolution. Genome Biol 2015;16(1). https://doi.org/10.1186/s13059-015-0585-4

[41] Wenner MS, Sullivan MA, Shah RN, Nadarad D, Grzybowski AT, Galat V, et al. Chromatin-immuno-precipitation of noncoding RNAs can act as cell-type specific activators of proximal gene transcription. Nat Struct Mol Biol 2017;24(7):596-603. https://doi.org/10.1038/nsmb.3424

[42] Schonherr K, Sall N-A, Ghaider K, Choveud E, Etemadifar M, et al. Integrative Analysis of IncRNAs in TH1 Cell Lineage to Discover New Potential Biomarkers and Therapeutic Targets in Autoimmune Diseases. Mol Ther Nucleic Acids 2018;12:393–404. https://doi.org/10.1016/j.omtn.2018.05.022

[43] Xu W, Zhao N, He X, Le H, N Chen Y, et al. Genome-wide identification and functional prediction of cold and/or drought-responsive IncRNAs in cassava. Sci Rep 2017;7(1): https://doi.org/10.1038/s41598-018-38462-x.

[44] Wang X, Yang C, Guo F, Zhang Y, Ju Z, Jiang Q, et al. Integrated analysis of network and long noncoding RNAs in the serum from Holstein bulls with high and low sperm motility. Sci Rep 2019;9(1). https://doi.org/10.1038/s41598-018-38462-x.

[45] Schulze BM, Gallicio GA, Cesaroni M, Lupey LN, Engel N. Enhancers compete with a long non-coding RNA for regulation of the Kcnq1 domain. Nucleic Acids Res 2018;46(10):1460–33. https://doi.org/10.1093/nar/gky1092

[46] Ø Padua D, Mahurkar-Joshi S, Law IKM, Polytarchou C, Vu JP, Pisegna JR, et al. A single-cell RNA-seq map of the human immune system. Nature 2014;511(7510):421–7. https://doi.org/10.1038/nature13595

[47] Li Y, Zhang D, Zhang Y, Xu X, Bi L, Zhang M, et al. Association of IncRNA polymorphisms with triglyceride and total cholesterol levels among myocardial infarction patients in Chinese population. Gene 2016;588:143684. https://doi.org/10.1016/j.gene.2016.11.014

[48] Li H, Han S, Qiu G, Yao Ye, Li S, Yuan C, et al. Long non-coding RNA CDKN2BAS1 reduces inflammatory response and promotes cholesterol efflux in atherosclerosis by inhibiting ADAM10 expression. Aging (Albany NY). 2017;9(9):1695–713. https://doi.org/10.18632/aging.101608

[49] Hubberten M, Bochenek G, Chen H, Häslar R, Wiehe R, Rosenstiel P, et al. Linear isoforms of the long noncoding RNA CDKNA2B-AS1 regulate the c-myc enhancer binding factor RBMS1. Eur J Hum Genet 2019;27(1):1–8. https://doi.org/10.1038/s41431-018-0210-7

[50] Bögevik E, Saadat KASM, Arman K, Bozyegik I, Ikeda M-A. Enhanced EZF1 activity increases invasive and proliferative activity of breast cancer cells through non-coding RNA CDKNA2B-AS1. Meta. Gene 2020;24:100691. https://doi.org/10.1016/j.gene.2020.100691

[51] van Dam S, Vosa U, van der Graaf A, Franke L, de Magalhaes JP. Gene co-expression analysis for functional classification and gene-disease predictions. Brief Bioinform 2018;19(4):575–92. https://doi.org/10.1093/bib/bby058

[52] Vavitch-Pachima Y, Higuchi K, Ikeda M-A, Iwata J, Komeno T, Kameyama T, et al. Discovery of autism/intellectual disability somatic mutations in Alzheimer’s brains: mutated ADNP cytoskeletal impairments and repair as a case study. Mol Psychiatry 2021;26(3):1619–33. https://doi.org/10.1038/s41386-020-00786-3

[53] Nativio R, Donahue G, Berson A, Lan Y, Amlie-Wolf A, Tuzer F, et al. Dysregulation of the epigenetic landscape of normal aging in Alzheimer’s disease. Nat Neurosci 2018;21(4):497–505. https://doi.org/10.1038/s41593-018-0221-0

[54] Sinivasan K, Friedman BA, Ettebrexia A, Huntley MA, van der Brug MP, Foreman O, et al. Alzheimer’s patient Microglia Exhibit Enhanced Aging and Unique Transcriptional Activation. Cell Rep 2020;30(13):107843. https://doi.org/10.1016/j.celrep.2020.107843

[55] Pantazatos SP, Huang Y-Y, Rosoklija GB, Dwork AJ, Arango V, Mann JH. Whole-transcriptome brain expression and exon-usaging profiling in major depression and suicide: evidence for altered glial, endothelial and ATPase activity. Mol Psychiatry 2017;22(5):760–73. https://doi.org/10.1038/mp.2016.130

[56] Labonté B, Engmann O, Purushothaman I, Menard C, Wang J, Tan C, et al. Sex-specific transcriptomic signatures in human depression. Nat Med 2017;23(7):795–803. https://doi.org/10.1038/nm.4312

[57] Choi S, Park HS, Cheon MS, Lee K. Expression profile analysis of human peripheral blood mononuclear cells in response to aging. Arch Immunol Ther Exp [Warsz] 2005;53(2):151–9.

[58] Wang L, Luan T, Zhou S, Lin J, Yang Y, Liu W, et al. LncRNA HCP5 promotes tumor growth, cancer progression and drug resistance by targeting miR-215a-3p. Cell Death Dis 2019;10(8):430–403. https://doi.org/10.1038/s41419-019-04235-9

[59] Béroueche-Jallon F, Gautier S, Lavoie-Chardon E, Sbarra L, Henry C, Madore A-M, et al. Identification of Synaptic Vulnerability Genes of Adult Asthma in French Canadian Women. Can Respir J 2016;2016:11–12. https://doi.org/10.1155/2016/4564341

[60] Chadha P, Mahurkar-Joshi S, Law I, Polytarchou C, Vu JP, Pisegna JR, et al. Long noncoding RNA signature for ulcerative colitis identifies IFRCN1-AS1 as an enhancer of inflammation. Am J Phys Gastrointest Liver Physiol 2016;311 (3):G446–57. https://doi.org/10.1152/ajpgi.00212.2016

[61] Züchner S, Gilbert JR, Martin ER, Leon-Guerrero CR, Xu P-T, Browning C, et al. The genetic architecture of late-onset Alzheimer disease families linked to 9p21.3. Ann Hum Genet 2008;72(6):275–31. https://doi.org/10.1111/j.1469-1809.2008.00474.x

[62] Zhang X, Chen S, Al-Mahrouq S, Al-Abdulmajeed SH, Borio J, Baranowa E, et al. Intrinsic Polymorphisms in the CDKN2B-AS1 Gene Are Strongly Associated with the Risk of Myocardial Infarction and Coronary Artery Disease in the Saudi Population. Int J Mol Sci 2016;17(3):395. https://doi.org/10.3390/ijms17030395

[63] Li Y, Zhang D, Zhang Y, Xu X, Bi L, Zhang M, et al. Association of IncRNA polymorphisms with triglyceride and total cholesterol levels among myocardial infarction patients in Chinese population. Gene 2016;588:143684. https://doi.org/10.1016/j.gene.2016.11.014

[64] Speake C, Skinner SO, Berel D, Whalen E, Durfort MJ, Young WC, et al. A composite immune signature parallels disease progression across TID subjects. JCI Insight 2019;4(23). https://doi.org/10.1172/jci.insight.126911.171207.1269117203.
[86] Herring BP, Chen M, Mihaylov P, Hoggatt AM, Gupta A, Nakeeb A, et al. Transcriptome profiling reveals significant changes in the gastric muscularis externa with obesity that partially overlap those that occur with idiopathic gastroparesis. BMC Med Genomics 2019;12(1). https://doi.org/10.1186/s12920-019-0550-3.

[87] Paczkowska-Abdulsalam M, Niemira M, Bielska A, Szalkowska A, Raczkowska BA, Junnila S, et al. Evaluation of Transcriptomic Regulations behind Metabolic Syndrome in Obese and Lean Subjects. Int J Mol Sci 2020;21(4):1455. https://doi.org/10.3390/ijms21041455.

[88] Shu Yi, Wang Yi, Lv W-Q, Peng D-Y, Li J, Zhang H, et al. ARRB1-Promoted NOTCH1 Degradation Is Suppressed by OncomiR miR-223 in T-cell Acute Lymphoblastic Leukemia. Cancer Res 2020;80(5):988–98. https://doi.org/10.1158/0008-5472.CAN-19-1471.

[89] Ji Q, Zhou L, Sui H, Yang L, Wu X, Song Q, et al. Primary tumors release ITGBL1-rich extracellular vesicles to promote distal metastatic tumor growth through fibroblast-niche formation. Nat Commun 2020;11(1). https://doi.org/10.1038/s41467-020-14869-x.