Research Paper: Bioinformatics Investigation and Contribution of Other Chromosomes Besides Chromosome 21 in the Risk of Down Syndrome Development

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ABSTRACT

Introduction: Down syndrome as a genetic disorder is a popular research topic in molecular studies. One way to study Down syndrome is via bioinformatics.

Methods: In this study, a gene expression profile from a microarray study was selected for more investigation.

Results: The study of Down syndrome patients shows specific genes with differential expression and network centrality properties. These genes are introduced as RHOA, FGF2, FYN, and CD44, and their level of expression is high in these patients.

Conclusion: This study suggests that besides chromosomes 21, there are additional contributing chromosomes to the risk of Down syndrome development. Besides, these genes could be used for clinical studies after more analysis.

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Highlights

- RHOA, FGF2, FYN, and CD44 significantly up-regulate hub-bottlenecks in patients with Down syndrome.

- Biological processes related to these top four genes could be disrupted.

- The introduced central genes could be considered as potential biomarkers after conducting validation studies.

- Other chromosomes than chromosome 21 contribute to the risk of Down syndrome development.

Plain Language Summary

Down syndrome as a genetic disorder is a popular research topic in molecular studies. One way to study Down syndrome is via bioinformatics. In this study, a gene expression profile from a microarray study was selected for more investigation. The study of Down syndrome patients shows specific genes with differential expression and network centrality properties. These genes are introduced as RHOA, FGF2, FYN, and CD44, and their level of expression is high in these patients. This study suggests that besides chromosomes 21, there are additional contributing chromosomes to the risk of Down syndrome development. Besides, these genes could be used for clinical studies after more analysis.

1. Introduction

Trisomy 21 is the most frequent chromosomal aberration seen in up to 1 in 700 population of newborns (Busch et al., 2005). Patients with this syndrome manifest vast abnormalities, such as metabolism dysfunction, dysmorphic characteristics, and mental retardation as the most highlighted one (Bajo, Fruehauf, Kim, Fountoulakis, & Lubec, 2002; Starbuck, Cole III, Reeves, & Richtsmeier, 2017). There are different detection approaches for the prenatal stage of this developmental disorder, each with its pros and cons (Busch et al., 2005). Most molecular studies are also done either via amniotic fluid or serum sample of pregnancies with trisomy 21 for diagnostic purposes (Busch et al., 2005; Tsangaris et al., 2006). However, to understand what triggers the broad range of abnormal phenotype in Down syndrome (DS) (Teeling et al., 2005), the molecular profile of patients with this defect must be explored. The mechanisms behind the phenotype of DS have been studied via different molecular approaches (Lockstone et al., 2007). Over the past decade, some investigations shed light on the complex mechanism of DS through large-scale analysis, including transcriptomic and proteomic studies (Bajo et al., 2002; Di Domenico et al., 2014; Lockstone et al., 2007). At these scales, information related to the expression profile of genes and proteins could be retrieved for any condition such as abnormal ones like a disease state (Rezaei–Tavirani, Tavirani, & Rostami, 2018).

Besides, bioinformatics can reveal different aspects of identified molecules by high throughput data analysis (Rezaei– Tavirani, Bashash, et al., 2018). The function of biomolecules is handled and mediated by interacting with other molecules (Zamanian Azodi et al., 2018). One of the identified novel processes related to the DS mechanism was the oxidative stress process, which was repeatedly pinpointed by many proteomic studies (Butterfield, Di Domenico, Tramutola, Head, & Perluigi, 2017; Di Domenico et al., 2014; Perluigi et al., 2011). Furthermore, this process associates with the transition of DS alone to DS with Alzheimer disease (Butterfield et al., 2017). Other molecular studies, such as a bioinformatics approach, suggest that the Down Syndrome Critical Region (DSCR) of chromosome 21 may have a tremendous regulatory impact on this disorder (Chen et al., 2018). Another network analysis that was also conducted on the current and in the combination of other microarray database indicated that there are some DEGs, including BCL2, HSP90 beta, UBX2, and TMEM50B, that might be important in DS (Zhao, Zhang, Ren, Zong, & Kong, 2016).

There are essential points such as centrality properties in an interacting profile (as a scale-free network) that influence the whole system. Centrality analysis is through the identification of two common parameters of degree and betweenness centrality. Any changes in central elements of a scale-free network could result in an abnormal phenotype such as a disease state (Zamanian-Azodi, Rezaei-Tavirani, Rostami-Nejad, & Tajik-Rostami, 2018). Therefore, identifying these essentials could

provide further information and validation of biomarkers linked to the disorder condition. In this respect, we applied Protein-Protein Interaction (PPI) network analysis to reveal this aspect of molecular features in disorders such as DS.

2. Materials and Methods

In this work, we investigated Differentially Expressed Genes (DEGs) with centrality features in a protein-protein interaction network pattern. For this aim, at first, we queried the Gene Expression Omnibus (GEO) database at the National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/geo/). It provides a gene expression profile of DS patients in comparison with healthy controls. A research carried out by Bahns et al. entitled "Expression profiling of human adult postmortem brain tissue from adult Down syndrome and healthy control subjects, Series GSE5390, platform: GPL96" (Lockstone et al., 2007) was selected for our bioinformatics evaluation. The chip used in this study was Affymetrix HGU133A GeneChips. The conductors of this study used RNA extraction from postmortem brain samples of 8 healthy subjects versus 7 Down syndrome types (dorsolateral prefrontal cortex) from female and male via microarray. The subjects' Mean (SD) ages for disease and control groups were 58.6 (9.4) years and 47.8 (10.8) years, respectively. At first, GEO2R online analyzer (https://www.ncbi.nlm.nih.gov/geo/geo2r/) in the GEO database analyzed these two groups. However, before that, cross-comparison via box plot analysis was carried out to determine the quality of the groups. If the samples are median-centered, then the procedure could be continued by identifying the top 250 ranked DEGs in DS.

The fold change threshold was obtained by evaluating the cumulative frequency of mRNA-expressed profile between study groups among 250 genes. A cutoff point of 2 for Fold Change (FC) was designated to detect the most DEGs among 250 tops. The correction test for the raw P value was set to Benjamini-Hochberg (False discovery rate) as the default option. The significance threshold that is acceptable for the DEGs is an adjusted P value of 0.05 or less. The uncharacterized DEGs among the analyzed significant DEGs were excluded, and the remained ones were applied for the construction of a PPI network via Cytoscape v. 3.6.1 (https://cytoscape.org/) and its plugin Search Tool Retrieval of Interacting Genes (STRING DB) (https://string-db.org/) (Shannon et al., 2003; Szklarczyk et al., 2016). Network Analyzer was used to determine the most critical nodes in the network in terms of interaction patterns (Assenov, Ramírez, Schelhorn, Lengauer, & Albrecht, 2007). Two

parameters were fundamental for this purpose: Degree (K) and Betweenness Centrality (BC). Nodes with the highest rank of these two features are called hub-bottlenecks (Safari-Alighiarloo, Rezaei-Tavirani, Taghizadeh, Tabatabaei, & Namaki, 2016).

Following the determination of hub-bottlenecks, CluePedia (http://www.ici.upmc.fr/cluepedia/) explores gene/protein/miRNA associations linked to the ClueGO networks with designated scores. Here, we examined the expression profile of these central nodes via extracting from the expression input file in the CluePedia panel. Depending on how many spots are available in the related GEO input file form for the corresponding gene, all will be extracted and merge as node labels (Bindea, Galon, & Mlecnik, 2013). Consequently, the most significant differentially expressed ones were then searched against ClueGO for enrichment and action type analysis (Bindea et al., 2013; Bindea et al., 2009). ClueGO is an application in Cytoscape that could analyze the functional properties of the queried genes. The enrichment analysis includes a Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) evaluations. Here the BP is examined for the significant DEGs. The action-type analysis between these genes was conducted via STRING Action File, V10.5., 2017., for 5 in-
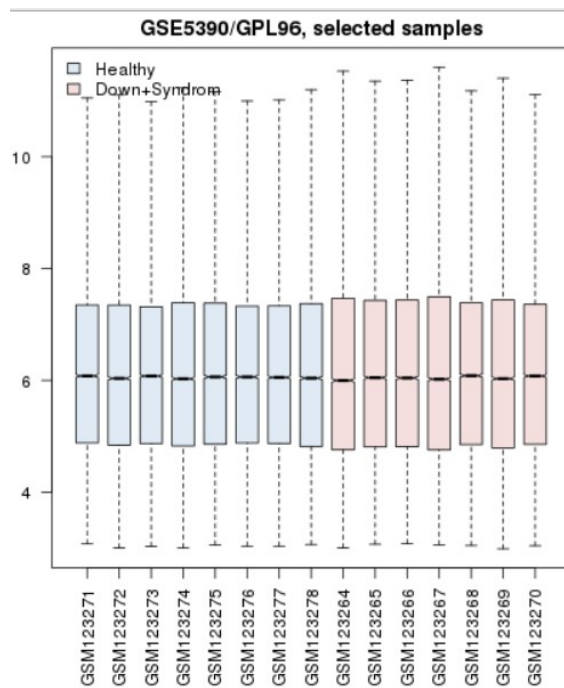
Figure 1. Graphical view of value distribution in 8 healthy and 7 DS cases via boxplot analysis. Blue color indicates control samples while pink color indicates DS samples

Lateral axis: Names of samples; Longitudinal axis: The genes.

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teraction enrichments: expression, activation, inhibition, post-translational modifications, and catalysis. To determine these interactions, kappa scoring (0-1) was used for scaling the edge strength, which is displayed by edge thickness. Moreover, this plugin can be used for gene ontology (biological process, molecular function, and cellular component) analysis. In a way, a network view of groups of terms associated with individual gene ontology could be provided. Our experiment covers one of the domains introducing biological process relationships. The term grouping for biological process strength was calculated by kappa statistics (this score is between 0-1), and the assigned cutoff was 0.5. This statistic measurement is used for the determination of grouping terms. The higher the k score, the lower the chance of grouping biological terms. Moreover, terms are grouped based on the default setting: number of genes in terms: 3 and percentage of genes contributing in terms: 4. Likewise, default options were applied for the levels of ontology as follows: minimum level of ontology as 3, the maximum level of ontology as 8. The correction method in this regard is Bonferroni step down (P≤0.05). The enrichment/depletion test: two-sided based on hypergeometric.

3. Results

Transcriptome analysis of DS versus healthy controls was taken from the GEO database via GEO2R. This comparison was first analyzed by boxplot to evaluate the quality of samples in gene expression (Figure 1).

The top 250 DEGs were determined, and then those with gene names and fold change of ≥ 2 were considered for network establishment by STRING. The range of significance level was from 0.0003 to 0.0026. A total of 65 up-regulated and 3 down-regulated genes (FC>2) were obtained. A network of up-regulated and down-regulated genes with the addition of 100 surrounding interacting ones was constructed. The network included a main connected component (contain 156 nodes and 3420 edges) and 12 ones that were not connected to the leading network (the data are not shown). For hub-bottleneck identification, the 20% of top genes with the highest amount of degree and betweenness centrality were recognized. A total of 19 common genes from 34 top ones based on K and BC were assigned as hub-bottlenecks (Table 1). To visualize and explore the expression profile of the hub-bottlenecks DS network, we used CluePedia (Figure 2). As depicted in Figure 2, except MBOAT4 (the low expressed gene), the other genes are differentially expressed in DS patients. However, only RHOA, FGF2,
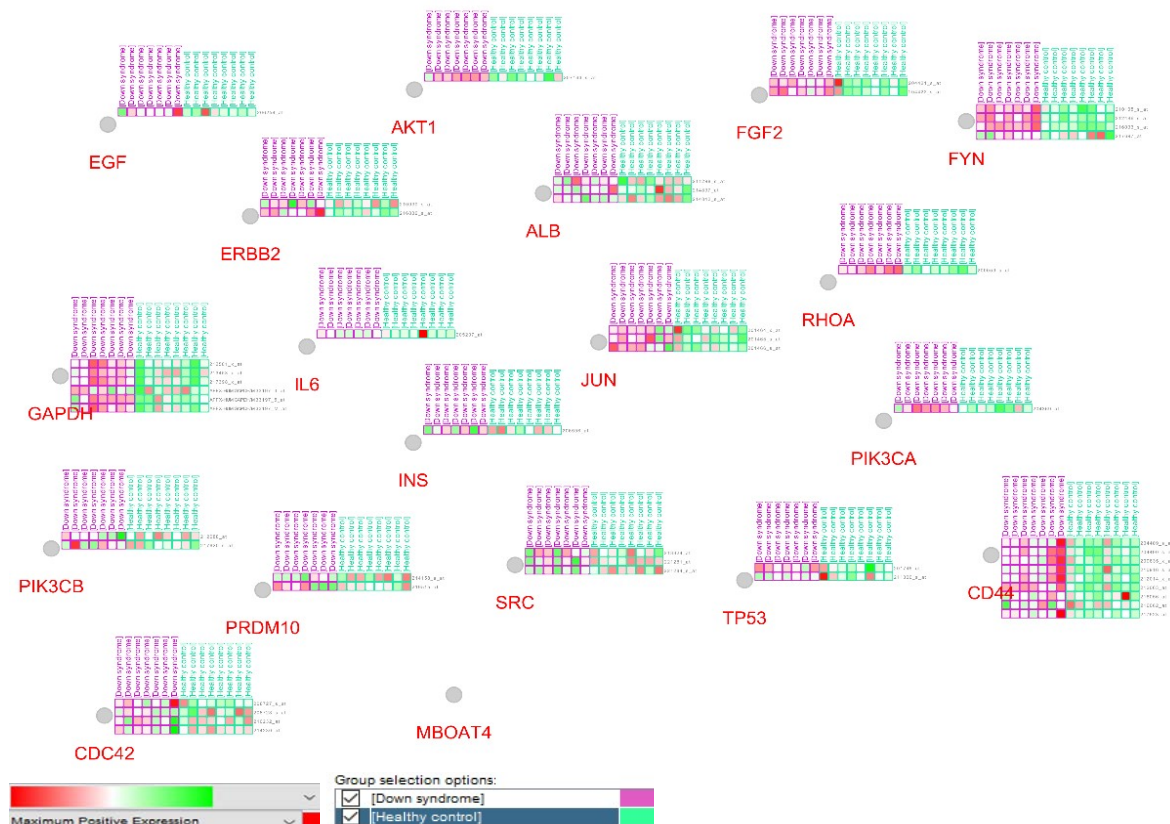

Figure 2. Normalized expression showing as node labels for 18 found genes using DS profile

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Table 1. The list of significant hub-bottlenecks that are differentially expressed in Down syndrome patients L

Row	Gene Name	Protein Name	Adjusted P	LogFC	Chr	Degree (K)	Betweenness Centrality (BC)
1	RHOA	Ras homolog family member A	0.002	0.8	3	73	0.01
2	FGF2	Fibroblast growth factor 2	0.030	0.9	4	76	0.02
3	FYN	FYN proto-oncogene, Src family tyrosine kinase	0.001	1.1	6	81	0.01
4	CD44	CD44 molecule (Indian blood group)	0.010	1.9	11	80	0.01

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FYN, and CD44 hub-bottlenecks are significant in differential expression (The criteria for assigning genes with more than one spot as significant differential expression ones was to have at least one spot with significant differential expression).

Statistical and centrality properties of these 4 significant DEGs are tabulated in Table 1. Besides, the same plugin, CluePedia, was used to gain further knowledge about the 4 significant DEGs in terms of interaction type (Figure 3). Activation, inhibition, catalysis, expression, and binding actions were determined.

CluePedia and CluGO did biological process analysis related to the RHOA, FGF2, FYN, and CD44 DEGs. As shown in Figure 4, 15 terms are identified, which are clustered in 4 classes.

In Figure 2, the data are extracted from an input file, GSE5390_series_matrix merging in the CluePedia application. The rows are the number of genes' spots in the sample considered Differentially Expressed (DE) probes. The color scheme from red (maximum positive expression) to green (maximum negative expression) shows positive to negative expression changes. White and grey refer to zero expression and missing values, respectively.

Several spots were available for FGF2, FRN, CD44, whereas one spot was extracted for RHOA.

Different colors indicate different action types; Green: Activation, Red: Inhibition, Purple: Catalysis, and Blue: Binding.

Four groups of biological processes are present here, namely negative regulation of blood vessel endothelial cell migration, epiboly, hyaluronan catabolic process, and regulation of adherents junction organization, in which the most highlighted one is the first mentioned term in blue color. These 4 genes are assigned to different terms. The node colors indicate what terms are linked

to that specific gene. For example, FYN is only related to one term, shown with that particular term color dark green. Number of Genes per term: 2, Gene percentage per term: 3%. Kappa Score: 0.5. The corrected method for P value was Bonferroni step down.

4. Discussion

Down syndrome as a genetic disorder is not well-studied in terms of molecular profiling (Liu et al., 2017). Identification of molecular events in DS could increase our understanding of its mechanisms and develop better management approaches. One of which is examining differentially expressed genes in DS via array profiling. Furthermore, protein network mapping of these abnormally expressed genes could offer further understanding of the molecular behavior in an interacting system. For this aim, the DS gene expression profile (GSE5390) was investigated via different online and offline analyzing tools. At first, the comparison of two groups of healthy and DS patients was conducted by box plot through GEO2R online analyzer (Figure 1). The comparison showed that the values are median-centered, and consequently, the groups are comparable in terms of expression; hence, the data is suitable for further investigations. Analysis of GEO2R results showed 63 up-regulated versus 3 down-regulated DEGs,
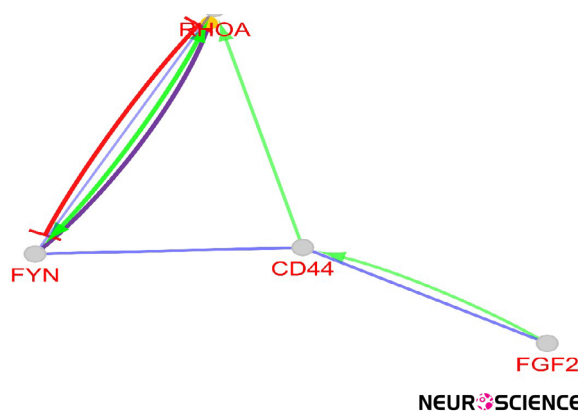
Figure 3. Action type analysis of the four significant central DEGs (differentially expressed genes) via CluePedia exploration

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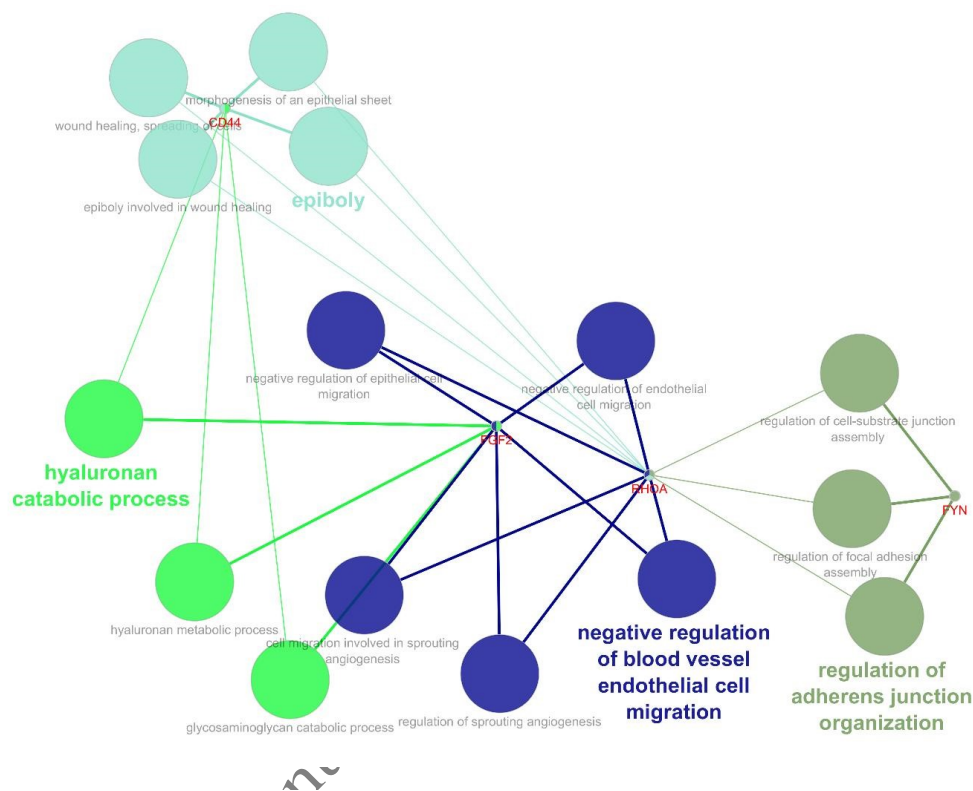
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Figure 4. Biological process examination of the four significant expressed hub-bottlenecks in Down syndrome via ClueGO+ CluePedia

which differentiate the DS group from healthy people. This fact correlates with previous reports that overexpression is dominant in chromosome 21 of the Down syndrome brain (Liu et al., 2017; Lockstone et al., 2007; Mao, Zielke, Zielke, & Pevsner, 2003).

Since PPI network analysis can screen the top DEGs to find more effective and influential genes relative to the onset and development of DS, the central elements (hub-bottlenecks) of the network were identified and tabulated in Table 1. A total of 19 genes were assigned as hub-bottlenecks for the DS network, in which none of them were among the top 250 DEGs except FYN. To evaluate the rank of these central genes in the DS expression profile, we applied CluePedia to combine the expression profile data with them, as shown in Figure 2. It can be inferred that all genes have expression values across the dataset GSE5390, except for MBOAT4. Since the DEGs which are expressed with at least one significant DE probe are considered as significantly expressed in DS, the INS, EGF, ERBB2, AKT1, ALB, GAPDH, IL6, JUN, PIK3CA, PIK-3CB, PRDM10, SRC, TP53, and CDC42 genes are not significantly expressed in DS. In other words, only 4 genes showed significant expression modifications among these 19 queried central genes. However, except RHOA, which

has one spot, the other 3 DE hub-bottlenecks (FGF2, FYN, and CD44) in some spots show inhomogeneous expression patterns. These 3 genes significantly and positively are expressed in patients but not in some individual samples. Overall, these genes are considered statistically significant in expression since they have at least one spot with Statistical Differential Expression SDE.

The 4 significant central DEGs include RHOA, FGF2, FYN, and CD44 with 1, 2, 4, and 9 rows, respectively. All of which are up-regulated and may propose that no down-regulated gene may have a role in DS phenotype and provide more evidence for the potential role of up-regulation in DS. Moreover, as indicated in Table 1, none of these genes is located on chromosome 21. They are, in fact, on chromosomes 3, 4, 6, and 11. This fact shows the effect of gene up-regulation events of other chromosomes in DS. Several experimental models in the last decades have shown that small GTPases of the Rho family are master regulators of the actin cytoskeleton in every cell type (Hall, 2005). This is important to know that these molecules have critical linkage in several features of the neuronal differentiation (Govek, Newey, & Van Aelst, 2005) and essential factors of many neurological syndromes and mental retardation (Newey, Velamoor, Govek, & Van

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Aelst, 2005; van Galen & Ramakers, 2005). Berto et al. (2007) showed that the protein TTC3, encoded by one of the central Down syndrome (Teeling et al. 2005) critical region candidate genes, physically interacts with citron kinase (CIT-K) and citron N (CIT-N). These two are effectors of the RhoA small GTPase that have previously reported to be involved in neuronal proliferation and differentiation (Berto et al., 2007).

As known, an enormous number of phenotypes, including learning difficulties, cardiac defects, unique facial features, and leukemia, accompany Down syndrome (Lana-Elola, Watson-Scales, Fisher, & Tybulewicz, 2011). FGF2 cooperates stromal cell support of normal hematopoiesis by modulating osteoblast functions in bone marrow (Sugimoto et al., 2016). So it could have an association with the leukemia of DS. Fyn is a tyrosine-specific phosphotransferase that is a member of the Src family of non-receptor tyrosine protein kinases (Resh, 1998). Ahmed et al. (2015) considered the role of FYN in the nuclear fraction of the hippocampus in DS models of rats (Ahmed et al., 2015). The CD44 is a cell surface marker involved in cell-cell interactions, adhesion, and migration and encoded by the CD44 gene on chromosome 11 (Spring et al., 1988). It is significantly associated with active Src family protein tyrosine kinases Fyn in plasma membrane domains of human lymphocytes (Ilangumaran, Briol, & Hoessli, 1998). Moreover, as shown in Table 1, FYN and RHOA are the most significantly expressed ones in DS. Additionally, CD44 showed the highest fold change among the DEGs.

The analysis continued by examining the action type between these prominent genes (Figure 3). As it is apparent, all the queried action types are present between FYN and RHOA. This phenomenon implies that these genes are in condensed interactions. These two genes, as indicated earlier, are the most significantly altered genes in DS as well. The other two genes, CD44 and FGF2, contributes to just two types of actions in this analysis, which is binding, and activation. To get a better view of the role of the critical 4 genes, CluePedia handled the enrichment analysis. As it is presented in Figure 4, these genes contribute to 4 classes of 15 biological terms. The expression changes of our genes could have an impact on these biological processes and, consequently, their dysregulation in DS. Negative regulation of blood vessel endothelial cell migration as the most highlighted group in our query shows that 2 genes of RHOA and FGF2 regulate this class of 5 biological terms. CD44 and FYN are involved in two and one groups of biological processes, respectively. In addition, RHOA is almost participating in all groups except in the hyaluronan

catabolic process. Therefore, RHOA may have distinctive roles in DS due to its vast molecular characteristics.

5. Conclusion

RHOA, FGF2, FYN, and CD44 (especially RHOA) and their related biological features may play indispensable associations in Down syndrome risk. Moreover, this study supports and suggests the fact that up-regulation may have more potential role in DS phenotype with a possible remarkable influence from other chromosomes significantly differentially expressed genes. However, more studies worth pursuing verification of this finding.

Ethical Considerations

Compliance with ethical guidelines

The data were available in a free access format from the GEO database.

Authors' contributions

Conceptualization and Investigation: Mona Zamanian Azodi and Mostafa Rezaei Tavirani performed study design and network analysis. Data collection and Data analysis and Writing – original draft: Majid Rezaei Tavirani and Mohammad Rostami Nejad.

Conflict of interest

The authors declared no conflict of interest.

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