

## Original Article

**Using Network Analysis to Examine the Brain Regions Connectivity of Functional Neurological Disorder Patient and Healthy Participant**Samira Ahmadi<sup>1\*</sup>, Mohammad Ali Oghabian<sup>2</sup>, Elham Faghihzadeh<sup>1\*</sup><sup>1</sup>Department of Statistics and Epidemiology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.<sup>2</sup>Department of Medical Physics and Biomedical Engineering, Tehran University of Medical Sciences, Tehran, Iran.

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## ABSTRACT

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FND disease;

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**Introduction:** Functional neurological disorder (FND) is one of the most common causes of neuropathy. However, its cause continues to be mysterious. Understanding the underlying mechanisms of that is crucial for treatment strategies. The study was conducted on brain images resting state fMRI taken from two volunteers (functional neurological disorder patient and healthy subject) who had the same characteristics.

**Methods:** We fitted Gaussian Graphical Models to a single subject data using a network approach.

**Results:** Based on the results of the networks, the number of significant edges was more in the left hemisphere in the patient, but in the healthy person, the number of these non-zero edges was more in the right hemisphere. Both the networks related to the healthy person and the patient had high density. Therefore, it indicated that the regions considered by these 2 people were strongly related to each other. The results showed the existence of more links and positive relationships between the regions, most of which showed a strong relationship. Among these connections, there were also negative connections. The networks of the healthy participant with almost symmetrical structures and the patient with Functional neurological disorder showed different characteristics, including asymmetry between the hemispheres.

**Conclusion:** this study is the first to demonstrate that the brain regions of both functional neurological disorder patient and healthy participant can be conceptualized as networks. The findings of this study add to a growing body of literature that functional neurological disorder patient brain regions can be analyzed using network approaches.

**Introduction**

Functional neurological disorder (FND) is one of the most common causes of neuropathy.<sup>1</sup> However, its cause continues to be mysterious.<sup>2</sup> also Functional disorders rank as the second

most frequent cause for seeking consultation with a neurologist, right after headaches.<sup>3</sup> The estimated incidence is approximately 12 cases per 100,000 people yearly. This suggests that there are approximately 8,000 new FND diagnoses annually in the UK, with an estimated

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50,000 to 100,000 individuals affected by the condition within the community.<sup>3</sup> Comprehending the mechanisms of FND is vital for enhancing diagnosis and treatment.

Functional MRI (fMRI) is used to study brain activity in disease states, with recent focus on assessing functional connectivity between brain regions.<sup>4</sup> This approach provides deeper insights into the cerebral mechanisms underlying various neurological conditions.<sup>5</sup> fMRI uses the BOLD signal, which is influenced by blood flow, volume, and the oxygenation ratio in brain regions. As a result, more active brain regions require increased oxygenated blood supply, leading to a stronger BOLD signal.<sup>6</sup> BOLD can be measured in the voxel<sup>7</sup> which represents the tiniest element within a three-dimensional image, akin to a pixel's role in a two-dimensional image.<sup>8</sup>

Graphical methods effectively visualize correlations and connections in three-dimensional fMRI data.<sup>9</sup> Among these models, undirected graphical models, in the form of network modeling of fMRI data, are extensively utilized.<sup>10</sup> fMRI's ability to assess brain regions over time is crucial for complex network strategies, leading to significant findings in neuroscience.<sup>4,11</sup> Lately, models of personality and psychopathology have incorporated networks of connections between thoughts, emotions, and behaviors.<sup>12</sup>

In the last five years, network research, particularly in psychology and fMRI sciences, has grown due to dynamic changes in nodes and edges.<sup>13</sup> Voxel-wise networks assign a node to each brain region (voxel) of equal size, utilizing fMRI time-series data for network creation. In contrast, anatomical methods use atlases to define nodes based on brain structure, where fMRI time-series data from

all voxels within a specific anatomical area are averaged to generate the network.<sup>14</sup> This study aimed to conduct to infer the brain connectivity network from time-dependent Resting state fMRI scans and to find the differences in brain communication according to fit the graphical lasso approach model on the data of FND patient and healthy person.

## Data Preparation and Preprocessing

DICOM images were converted to the user-friendly NIFTI format and processed using FSL 6.0.3 on Linux.<sup>15</sup> FSL is a widely used software for processing and analyzing neural data.<sup>16</sup> It's pertinent to acknowledge that the human brain possesses an innate symmetry in its structure. In greater detail, it consists of two cerebral hemispheres, each with corresponding spatial regions of interest (ROIs) on the opposite side, meaning every spatial ROI in the left hemisphere has an equivalent ROI in the right hemisphere. fMRI time series were recorded in 70 spatial ROIs, grouping close, similar voxels.

## Materials and methods

### Study design and setting

This study was of a Single-subject research type.<sup>7</sup> The study used rs-fMRI brain images from two volunteers with matching age, gender, and handedness. Both were men with 40 years old and right-handed. One of these two people was healthy and the other was diagnosed with FND. The data was collected from the data set recorded by the neuroimaging and analysis group at RCMCI of Tehran University of Medical Sciences. The data collection tool

included Siemens 3 Tesla MAGNETOM Prisma MRI machine.

### Statistical analysis

Indeed In such networks, many edges may be spurious. More nodes can cause overfitting and unstable estimates.<sup>13,17</sup> Statistical regularization techniques like least absolute shrinkage and selection operator (LASSO) are used to control spurious relationships in complex models. It involves a tuning parameter and shows good performance in estimating partial correlation networks.<sup>13</sup> A tuning parameter  $\gamma$ , which determines the level of sparsity, was set to 0.1 for our analysis. Graphical Lasso (glasso) is favored for its easy implementation and flexibility with noncontinuous data.<sup>18</sup> Consider the graphical lasso estimator:

$$\hat{\theta}^{gl} = \arg \min_{\theta} \{-\log \det(\theta) + \text{tr}(S\theta) + \lambda \|\theta\|_1\}$$

Where S is the sample covariance matrix.  $\lambda \geq 0$  and  $\|\theta\|_1$  is the sum of the absolute values of the elements of  $\theta$ . the covariance matrix is  $\Sigma$ , then the inverse covariance matrix  $\Theta = \Sigma^{-1}$  is called precision matrix.<sup>19, 10</sup>

The optimal network model is often determined by minimizing the Extended Bayesian Information Criterion (EBIC),<sup>20</sup> effective in identifying the true network structure.<sup>21</sup> Not all network nodes share equal importance in shaping its structure or influencing dynamic characteristics. Certain nodes play more crucial roles in these aspects.<sup>22</sup> Centrality indices measure a node's significance based on its connections. In network analysis, these indices model or predict processes like flow through a node or the network's resilience to node removal.<sup>12</sup> Key centrality measures such

as strength, betweenness, and closeness, along with network density measures were used to assess links. Each measure relies on a unique assumption to identify the most influential node. Strength centrality represents the total direct connections of a node. Betweenness centrality counts the shortest paths passing through a node, while closeness centrality sums the shortest paths from the node to all others in the network.<sup>23</sup> Afterward, to strengthen the replicability and generalisability of the findings, we examined the accuracy and stability of the network model following the recommendations of Constantini and Perugini and Epskamp.<sup>24-26</sup> The Fruchterman-Reingold layout algorithm was used to position regions based on connection strength, identifying significant and well-connected regions.<sup>27</sup> The networks represent Gaussian graphical models.<sup>12,28</sup> Every region is a node or vertex. And the partial correlation between any two nodes is represented as an 'edge'. Blue edges symbolize positive associations, while red edges indicate negative associations. The width and saturation of the edges indicate the strength of the partial correlations; wider and more saturated edges represent stronger correlations. The thickest links in the network correspond to the maximum value of the strongest edge.<sup>13</sup> The glasso algorithm incorporates a tuning parameter to control the network's sparsity, which we determined by minimizing the extended Bayesian information criterion (eBIC).<sup>18</sup>

$$\text{EBIC} = -2L + E \log(N) + 4\gamma E \log(P)$$

The EBIC employs a hyperparameter  $\gamma$  (gamma) to regulate the extent to which it prioritizes simpler models.<sup>20</sup> L represents the log-likelihood, N is the sample size, E is the number of non-zero edges, and P is the

number of nodes. It's crucial to distinguish the hyperparameter  $\gamma$  from the LASSO tuning parameter  $\lambda$ , and it must be manually specified. Typically, it is set within the range of 0 to 0.5.<sup>21</sup> This method has demonstrated significant efficacy in accurately identifying the true network structure.<sup>21</sup> The networks display all edges that survive regularization, ensuring that only statistically significant edges are retained in the network.<sup>29</sup> Centrality index stability was evaluated using case-dropping bootstrap on data subsets, testing stability, and associating new values with the original data.<sup>26</sup> In our sample, it is compared to 100 bootstrap samples. JASP (JASP Team, 2023) (Version 0.17.2) [Computer software] was used to determine the structure of the brain regions for visualization. Also, packages that can be used in R software include: bootnet, glasso, huge, mgm.

## Result

In this study, we considered 70 regions of the brain according to the Desikan atlas. The data matrix is a 70 x 144 matrix for each person. Regions considered in the Desikan-Killiany Atlas and their names are listed in Table 1. Network structure and centrality measures analysis

The estimated network structure is graphically depicted in Fig.1 and Fig.2 for the brain regions and the connection between them for the left and right hemispheres for the two people under study. Each hemisphere contains 35 nodes (Regions). According to the analysis, the thickness and color of the edges were different between the variables. The FND patient had 398 and 319 non-zero edges in the left and right hemispheres, respectively. The healthy participant had 354 and 393 out of 595 possible edges. Due to the large number of positive and negative edges, it is not possible to check them

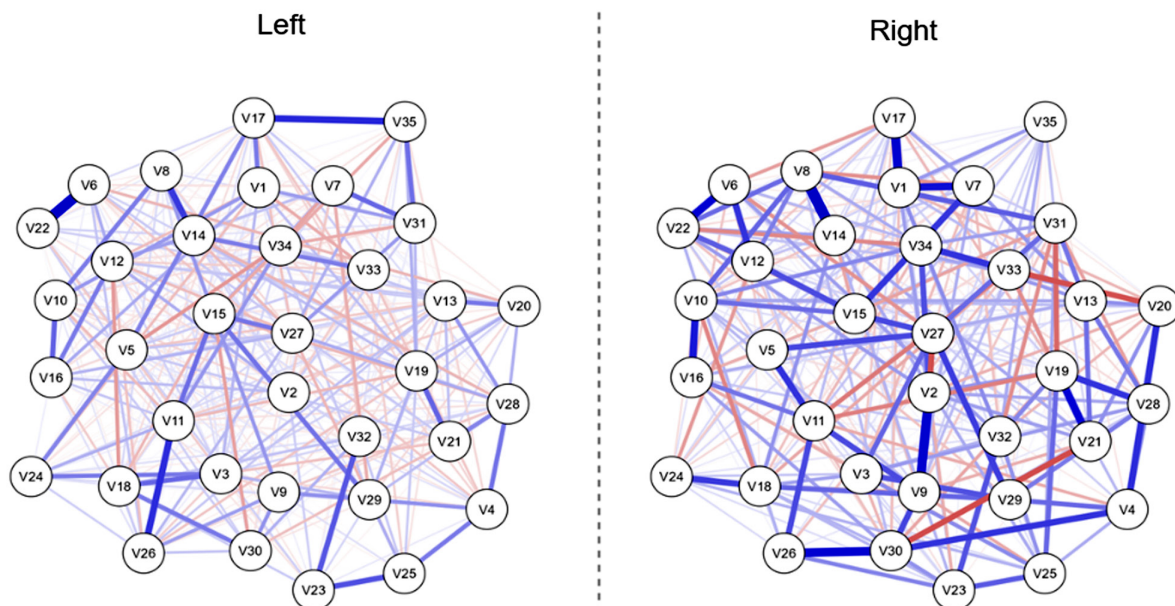


Figure 1. Estimated network structure of the left and right hemispheres for the FND patient

Note: Estimated network structure. Stronger correlations and weaker correlations were represented by thicker edges and thinner edges, respectively. Blue lines indicate positive edges and red lines indicate negative edges.

Table 1. Regions considered in the Desikan-Killiany Atlas and their names

| ID | DK Region Name                    | Hemispheres  |
|----|-----------------------------------|--------------|
| 1  | white_matter                      | Left , Right |
| 2  | Banks_superior_temporal_sulcus    | Left , Right |
| 3  | caudal_anterior_cingulate_cortex  | Left , Right |
| 4  | caudal_middle_frontal_gyrus       | Left , Right |
| 5  | corpus_calosum                    | Left , Right |
| 6  | cuneus_cortex                     | Left , Right |
| 7  | entorhinal_cortex                 | Left , Right |
| 8  | fusiform_gyrus                    | Left , Right |
| 9  | inferior_parietal_cortex          | Left , Right |
| 10 | inferior_temporal_gyrus           | Left , Right |
| 11 | isthmus-cingulate_cortex          | Left , Right |
| 12 | lateral_occipital_cortex          | Left , Right |
| 13 | lateral_orbitofrontal_cortex      | Left , Right |
| 14 | lingual_gyrus                     | Left , Right |
| 15 | medial_orbitofrontal_cortex       | Left , Right |
| 16 | middle_temporal_gyrus             | Left , Right |
| 17 | parahippocampal_gyrus             | Left , Right |
| 18 | paracentral_lobule                | Left , Right |
| 19 | pars_opercularis                  | Left , Right |
| 20 | pars_orbitalis                    | Left , Right |
| 21 | pars_triangularis                 | Left , Right |
| 22 | pericalcarine_cortex              | Left , Right |
| 23 | postcentral_gyrus                 | Left , Right |
| 24 | posterior-cingulate_cortex        | Left , Right |
| 25 | precentral_gyrus                  | Left , Right |
| 26 | precuneus_cortex                  | Left , Right |
| 27 | rostral_anterior_cingulate_cortex | Left , Right |
| 28 | rostral_middle_frontal_gyrus      | Left , Right |
| 29 | superior_frontal_gyrus            | Left , Right |
| 30 | superior_parietal_cortex          | Left , Right |
| 31 | superior_temporal_gyrus           | Left , Right |
| 32 | supramarginal_gyrus               | Left , Right |
| 33 | frontal_pole                      | Left , Right |
| 34 | temporal_pole                     | Left , Right |
| 35 | transverse_temporal_cortex        | Left , Right |

completely, Therefore we found some positive edges among the nodes under investigation. The strongest positive associations in Fig.1

the Left hemisphere emerged between Cuneus Cortex and Pericalcarine Cortex then there were other strong and positive edges between

Parahippocampal gyrus and Transverse Temporal Cortex, between Isthmus-cingulate cortex and Precuneus cortex and between Fusiform gyrus and Lingual gyrus. In Fig.1 the Right hemisphere the strongest positive links were between Fusiform gyrus and Lingual gyrus, between White matter and between Parahippocampal gyrus, between Banks of superior temporal sulcus and Inferior parietal cortex, between Precuneus cortex and Superior parietal cortex. Pars orbitalis and Frontal pole, Pars triangularis and V30 (Superior parietal cortex) in the Left hemisphere had negative correlations.

In Fig.2, the Left hemisphere exhibited the strongest positive links, particularly between Postcentral gyrus and Precentral gyrus, between Superior parietal cortex and Inferior parietal cortex. Additionally, in the Right hemisphere strongest and most positive connections were observed between Pars Opercularis and Pars triangularis, Between Precentral gyrus and

Postcentral gyrus, Between Superior temporal gyrus and Middle temporal gyrus, Between Temporal pole and Cuneus Cortex regions. Moreover, the most prominent negative relationship was observed between nodes Cuneus Cortex and Rostral anterior cingulate cortex.

In the left and right hemispheres in network centrality indices, Rostral anterior cingulate cortex and also in Fig.4, Pericalcarine cortex, Pars triangularis, Inferior temporal gyrus and Caudal middle frontal gyrus in the Left hemisphere and also Lateral occipital cortex, Superior temporal gyrus, Supramarginal gyrus and Inferior temporal gyrus of the Right hemisphere have the highest values of indices with respect to strength, closeness and betweenness. These regions had strong connections to nearby nodes, which means that they were more influential and important, and also played an important role in the network, and their activation had the greatest impact on

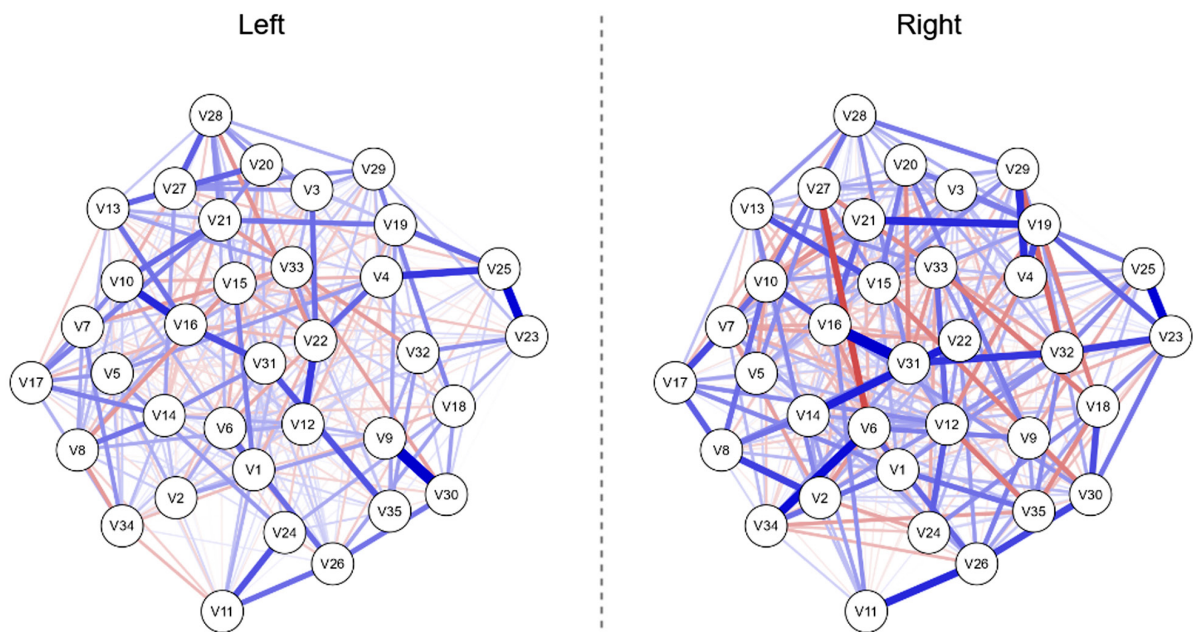


Figure 2. Estimated network structure of the left and right hemispheres for the healthy participant

other network nodes. Also, the access speed They had more connections to other nodes and exchanged information with other nodes in a shorter period of times. The results of the reduced bootstrap analysis showed that the average correlation between the centrality of the strength estimated in the original sample and the strength estimated in a random subsample preserves only a certain part of the cases (from 90% to 10%). Higher values indicate better stability of centrality estimates. The strength centrality and the closeness index are more stable than the betweenness index. In both graphs, in the right hemisphere, all three

indices are very close to each other, and the centrality indices showed a large correlation value (<0.05) even for a sample of 30% of the original dataset. But in the left hemispheres, the closeness index decreased steadily. An instability pattern is not uncommon in network analysis.

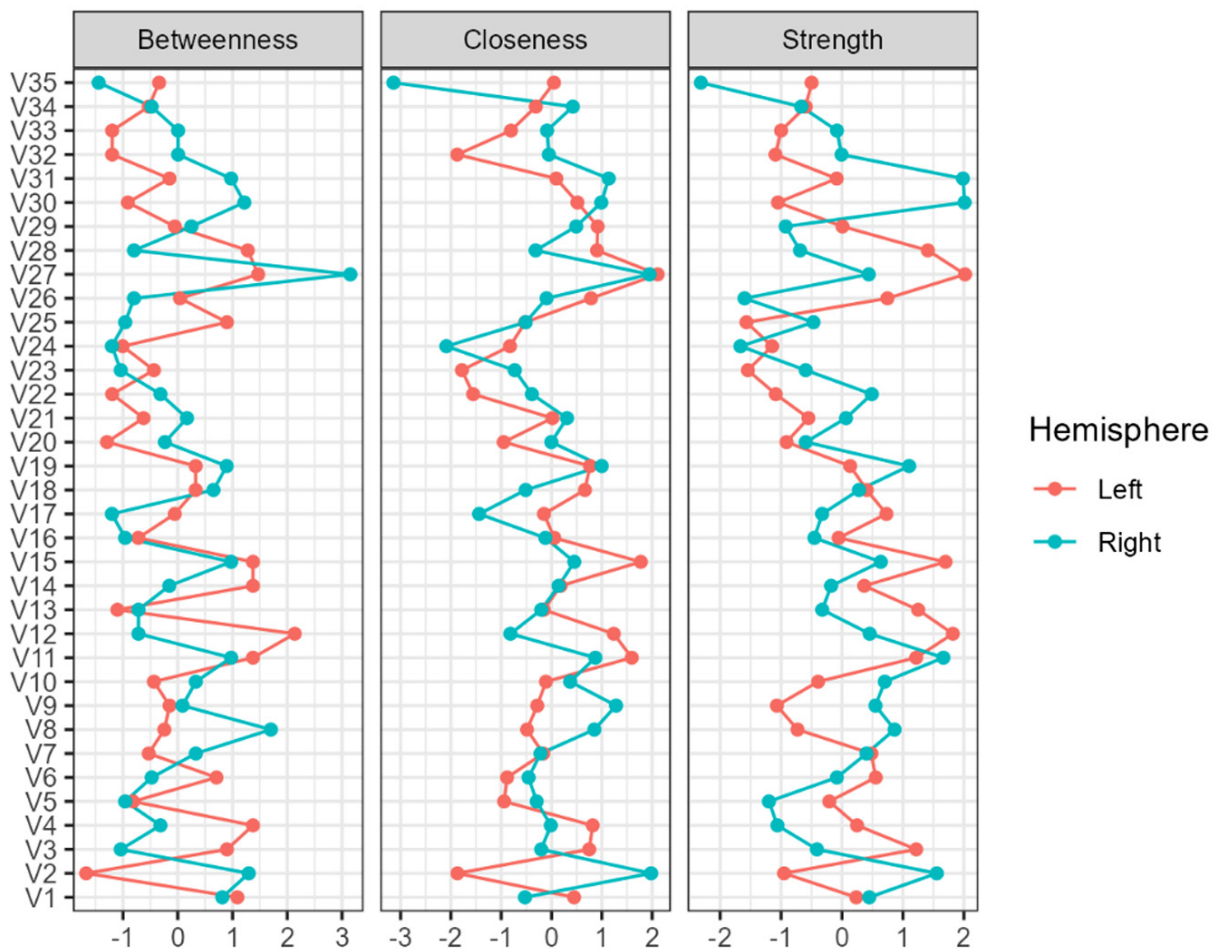


Figure 3. Standardized estimates of the centrality indices for FND patient  
 Note: Nodes with higher centrality are situated to the right from the vertical axis.

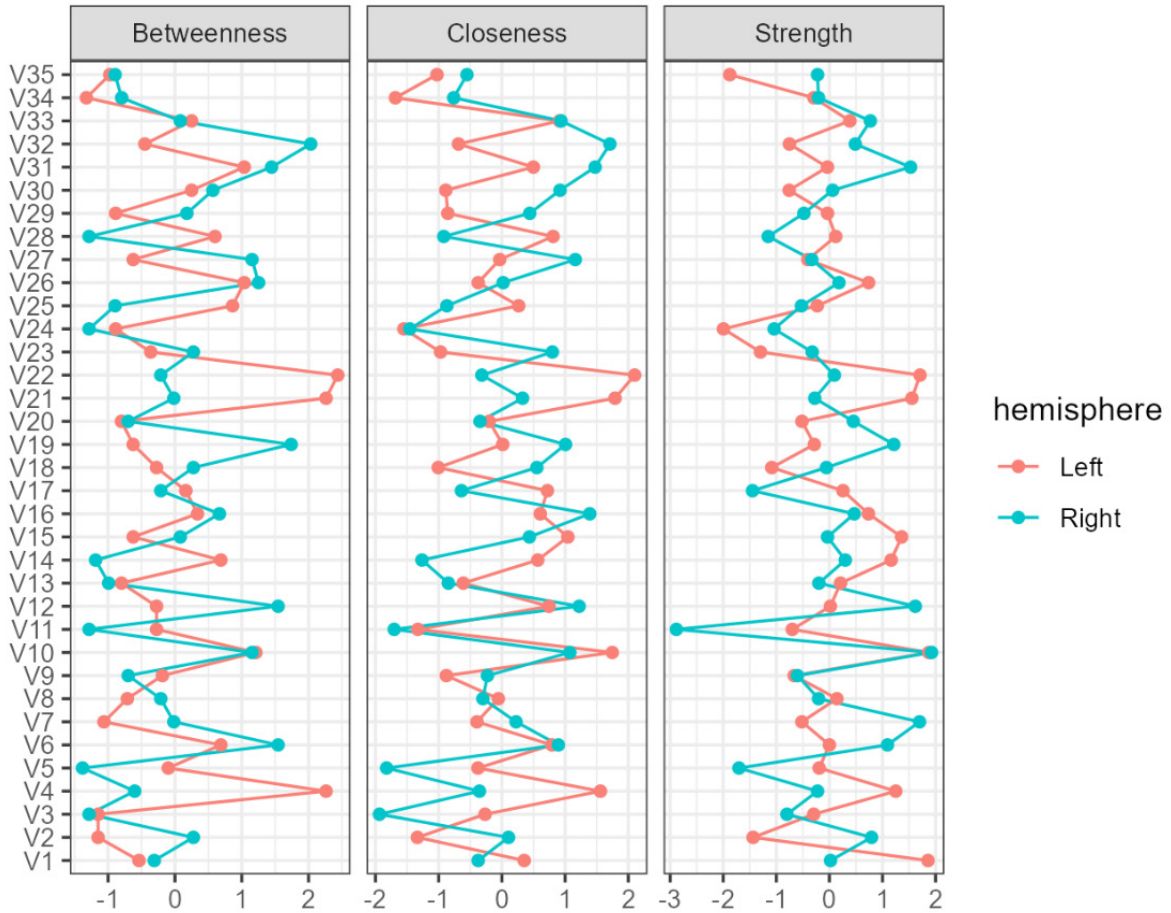


Figure 4. Standardized estimates of the centrality indices for healthy participant

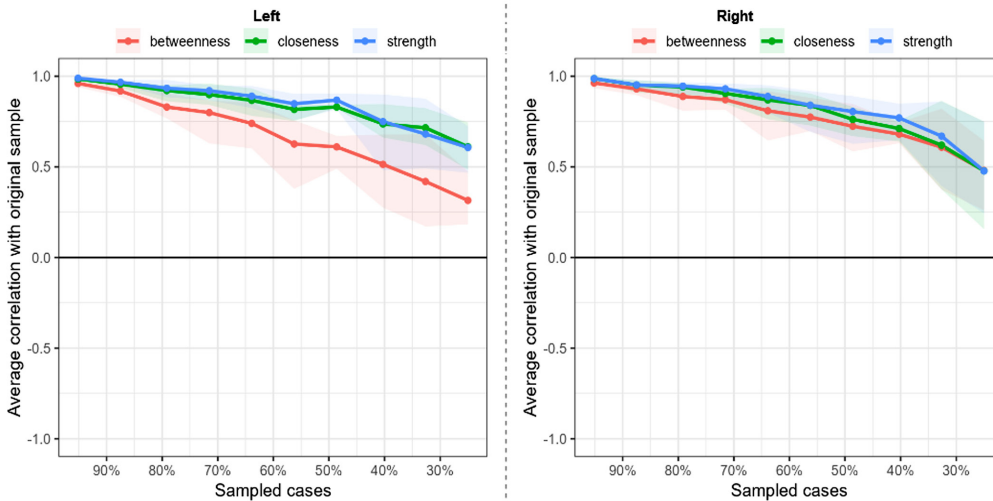


Figure 5. The stability of centrality indices for the FND patient using a case-dropping subset bootstrap approach  
 Note: The x-axis depicts the percentage of cases from the original sample used at each step. The y-axis shows how centrality indices of the initial network correlate with those of networks re-estimated after dropping more cases. Each line on the graph illustrates the correlations of strength, betweenness and closeness, while the areas represent the 95% confidence intervals(36).



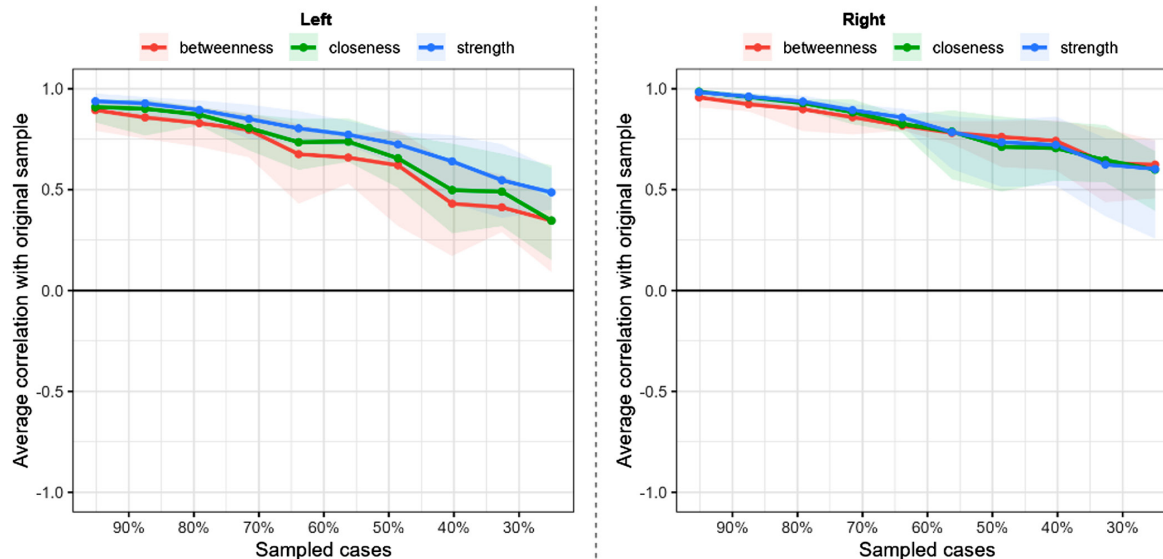


Figure 6. The stability of centrality indices for the healthy participant using a case-dropping subset bootstrap approach

## Discussion

To the best of our knowledge, this is the first study to describe the brain connectivity networks from resting-state fMRI scans of FND patient and healthy participant. Therefore the main objective of this study was to investigate the connection between 70 regions in both hemispheres of the brain of FND patient compared to healthy participant using a network approach. In this regard, our study was primarily exploratory in its approach. Networks were estimated using the EBICglasso method and graphical lasso for a Gaussian graphical model (GGM). In the patient, the number of significant edges was more in the left hemisphere. This is while the number of non-zero edges in the right hemisphere was higher in a healthy participant. Both the networks related to the healthy participant and the patient had high density. Therefore, it indicated that the regions considered in these two people were strongly related to each other. The results of the analysis showed the existence of more links and positive relationships between

the regions, most of which showed a strong relationship. Among these connections, there were also negative connections. Our findings revealed variations in the closeness, strength, and betweenness measures among different regions.

In the present study, the healthy subject displayed brain functional networks almost with efficient and symmetrical structures, consistent with several previous studies. However, the patient with FND exhibited different properties, including lower connectivity strength and asymmetry between hemispheres compared to the healthy subject, which is consistent with Yong Liu et al.<sup>30</sup> study on schizophrenia. Large-scale international studies examining patient experiences of FND have not been conducted except the study by Matt butler et al. (2021).<sup>31</sup> Sun et al. (2017) implemented anatomical weighted hemispheric brain networks for schizophrenia patients. Their results showed evidence of asymmetry in schizophrenic patients.<sup>32</sup> Their findings were consistent with previous observations from studies by Liu et al.<sup>30</sup> and the

structural network of Wang et al.<sup>33</sup> and Ottet et al.<sup>34</sup> Sascha Epskamp et al. conducted a study to investigate the relationship between brain structure and depression symptoms, focusing on exploring the symptoms of depression. In their brain symptom network, all brain structures were positively correlated. The Insula region showed a negative correlation with loss of interest in sex and sadness, while the Hippocampus region displayed a negative correlation with sadness and appetite changes and a positive correlation with loss of interest. The Cingulate region exhibited a negative association with sadness, and the Fusiform gyrus showed a weak but positive association with self-criticism and crying.<sup>35</sup>

We hope that the findings presented in this study can serve as a foundation for future research endeavors involving larger sample sizes in network analyses within this domain. This would mark a significant step toward validating and advancing such studies.

### **Strengths and limitations**

Despite these novel findings and implications of the study, the study is not without limitations and it is essential to recognize and take into account certain limitations. First, The study was of a single subject type and included only two people, which limits the generalizability of the findings. Therefore a large number of samples is needed to achieve higher statistical power to detect significant differences. Second, the method proposed in our study assumes that the brain connectivity network is stable over time, which may not be true in all cases. The study lacks a comprehensive comparison with other existing methods for inferring brain connectivity networks. The paper provides

a tool for neuroimaging researchers to better understand brain network structure in FND patients versus healthy individuals.

### **Conclusion**

In this research, a glasso model was used, which has been proposed for single-subject analysis. this study is the first to demonstrate that the brain regions of both FND patient and healthy participant can be conceptualized as networks. Our findings contribute to the expanding literature suggesting that FND patient data can be analyzed using network approaches. There is ample opportunity for further advancement in identifying the active regions of the brain in patients with FND using resting-state functional magnetic resonance imaging (rs-fMRI). Further research is required to explore the dynamic function of FND patients and the interconnections between brain regions, as well as to compare them with healthy participants. The graphical lasso method has extensive applicability, going beyond learning multiple networks to encompass other data structures, such as spatial or temporal data. It can be utilized to reveal gene regulatory network structures or identify patterns of brain activity across various regions and time points.

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## Declarations

Ethics approval and consent to participate  
The institutional review board of Zanzan university of medical sciences approved the protocol of the study (Ethics code : IR.ZUMS.REC.1400.456 ). The participants' privacy was preserved. All participants filled and signed the informed consent and assent.

## Consent for publication

'Not applicable.'

## Availability of data and materials

The data that support the findings of this study are available from EF, but restrictions are applied to the availability of these data, which were used under license for the current study, and are not publicly available. Data are, however, available from the authors upon reasonable request by EF and SA.

## Competing interests

The authors declare that there is no conflict of interest.

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## Authors' contributions

All authors read and approved the final manuscript. MO conceived the study and participated in the data collection. SA, EF participated in the data analysis and manuscript

preparation.

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