

Supplementary Analysis 1: Selection of the optimal number of clusters for Behavioral Tractography Analysis

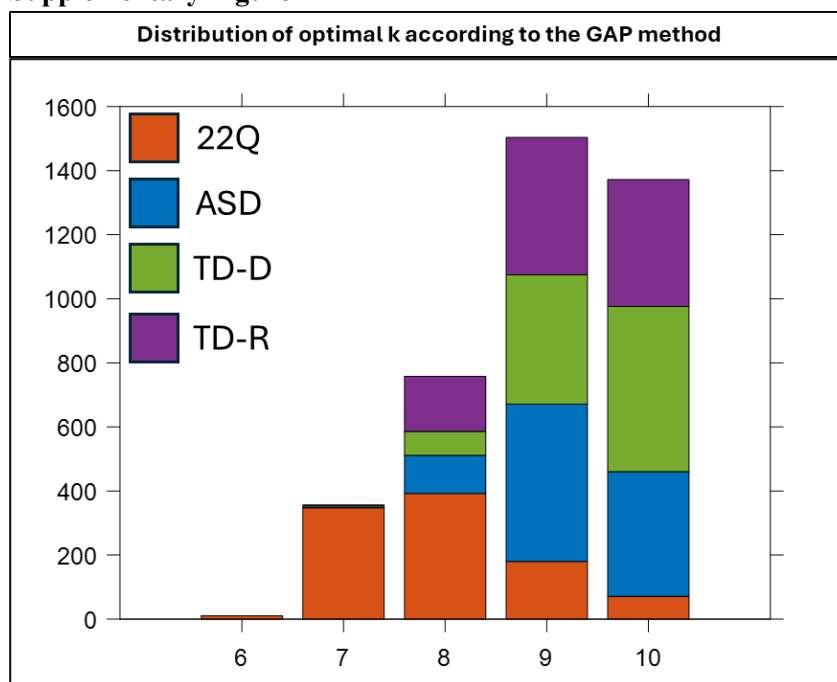
Methods

The optimal number of clusters was initially defined in each population separately. To this end, we employed the Gap algorithm implemented in the GapEvaluation Matlab function (<https://ch.mathworks.com/help/stats/clustering.evaluation.gapevaluation.html>), requesting evaluations for cluster counts ranging from 2 to 10. To address the potential ambiguity inherent in K-means clustering, due to the initial centroid selection, we applied the Gap criterion to 1000 versions of the K-means algorithm, varying the randomization used for initial centroid selection at each iteration. We utilized the K-means++ algorithm as the default method for initial centroid selection in Matlab's K-means implementation. This approach yielded a distribution of optimal K values, accounting for the non-deterministic nature of k selection and the impact of the initial centroid choice on the final output. Subsequently, we compared the results obtained for each population. To facilitate interpretability of differences in network structure across population, which was the essential aim of the BT procedure, we defined a single optimal number of clusters across all four populations, which was defined as the median of optimal K values computed in the 4 populations.

Results

Our analysis revealed distinct optimal cluster numbers for each population: 8 clusters for individuals 22q11.DS, 9 clusters for ASD, 10 clusters for TD-D, and 9 clusters for TD-R, which yielded a median value of 9 clusters across populations. The distribution of optimal K values computed across 1000 iterations in each population is displayed in Supplementary Figure 1.

Supplementary Figure 1



Distribution of optimal K values according to Gap criterion computed in each population across 1000 iterations of the k-means algorithm, varying the initial distribution of centroids at each iteration.

Supplementary Analysis 2: Replication of Behavioral Tractography across Typically Developing Discovery and Replication Samples.

In this analysis, we evaluated the replicability of the K-means clustering procedure employed to define Behavioral Tractography bundles across Typically Developing Discovery and Replication samples (TD-D and TD-R).

We firstly estimated the similarity of K-means clustering results, in terms of the extent to which paths were assigned to the same clusters in the TD-D and TD-R samples.

To this end we employed the Rand Index (RI), a widely used measure to assess the similarity between two sets of clustering results. The RI quantifies the level of agreement between the cluster assignments by evaluating the proportion of pairwise agreements and disagreements with. RI values can range from 0 to 1, where 1 indicates perfect concordance in clustering results.

We computed the RI between TD-D and TD-R, which we then compared against a null distribution obtained by permuting the TD-R clustering 1000 times, see Figure 1.

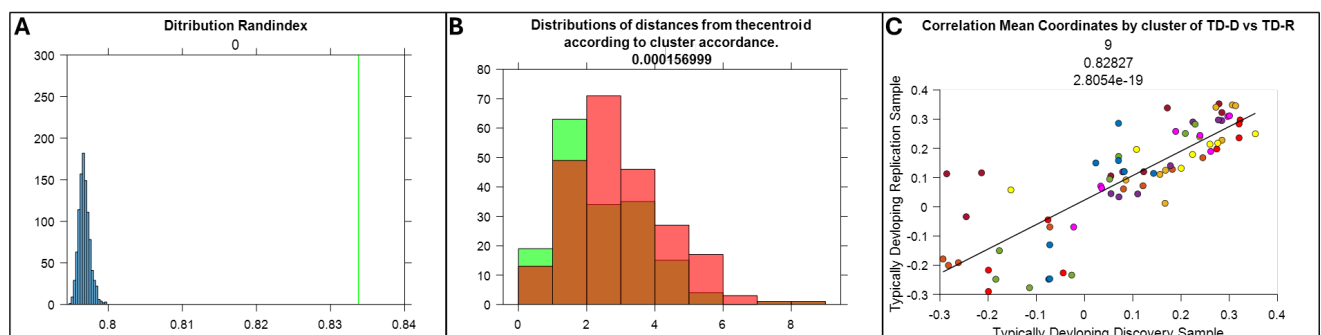
We also evaluated whether the correct attribution of paths to a given cluster across the two populations was moderated by the Euclidian distance between the path and the centroid of the corresponding cluster, which reflects the extent to which the trajectory of a given path is reflected in by the average trajectory of the corresponding BT bundle. We tested whether paths that more tightly reflected the average trajectory the BT bundle had a higher likelihood to be consistently assigned to the same cluster across populations, which would further validate the consistency of BT results across populations. see Figure 3.

After assessing the replicability of the composition of paths attributed to each BT cluster, we evaluated the qualitative similarity in the 3D trajectories of the resulting BT bundles. As described in the main text, the trajectory of each BT bundle was codified by the X and Y coordinates of the TL1-Starting-Point, TL1-Exit-Point, TL2-Entry-Point and TL2-End-Points. We correlated such XY coordinates across populations to estimate the qualitative similarity in the 3D trajectory of BT bundles.

Results

Overall, the K-means clustering procedure yielded highly consistent results with a Rand Index of 0.832 that was significantly higher than would be expected by chance $p < 0.001$. (See Supplementary Figure 2 Panel A). Moreover, paths that were inconsistent cluster assignment across populations also had a significantly higher Euclidian distance from their corresponding cluster centroids ($p < 0.001$). See Figure 2 Panel B. This would indicate that paths that more strongly contribute to the average trajectory of the corresponding BT-Bundle had also a higher likelihood to be consistently assigned across samples, which further validates the replicability of BT analysis across samples. Finally, the average trajectory of BT bundles was highly consistent across populations as indicated by the strong positive correlation of the average XY coordinates of BT bundles across populations ($R = 0.82$, $P < 0.001$). See Figure 2 Panel C.

Supplementary Figure 2:



Replication Behavioral Tractography Analysis across Typically Developing Discovery and Replication Samples.

Panel A: Empirically observed RandIndex value measuring the correspondence of K-means clustering results across TD-D and TD-R samples represented in green, compared against the null-distribution of the Randindex values computed across 1000 randomly permuted clustering solutions, represented in blue.

Panel B: Distribution of Euclidian distances between the trajectory of paths and the centroid of the cluster they were assigned to, for paths that were consistently assigned to the same cluster in TD-D and TD-R samples, represented in Green, and paths that were inconsistently clustered. A T-Test detected a significant difference between the two distributions ($p < 0.0001$) indicating that paths that more tightly resembled the average trajectory of their corresponding bundle were more likely to be consistently clustered across populations, overall validating the consistency of BT analysis across samples.

Panel C: Correlation of average 3D coordinates, characterizing the average trajectory of the 9 BT-Bundles across independent TD-D and TD-R sample.

Supplementary Analysis 3: Replication analysis in a subsample of High-IQ 22q11DS individuals

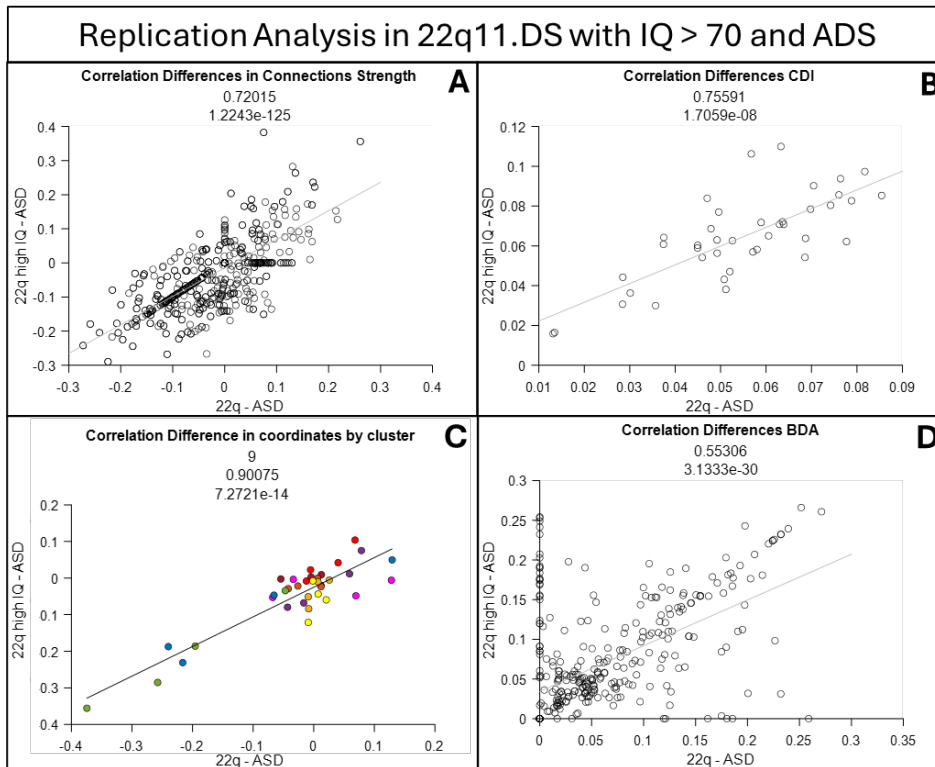
Methods: To verify that network differences observed across clinical samples were not entirely related to the higher prevalence of intellectual disability in the 22q11DS, we conducted supplementary analysis considering only individuals with Full-Scale-IQ > 70 , described demographically in Supplementary Table 1.

We compared the High-IQ-22q11DS sub-sample, to ASD with regards to the Connection Strength of individual connections, Connectivity-Dissimilarity-Index (CDI) of individual variables, average trajectory of Behavioral-Tractography (BT) Bundles and Behavioral Diffusion Analysis (BDA) of individual variables. We employed the same permutation-testing procedure described in the main text to test statistical significance of differences across samples which we describe in detail in Supplementary Table 1. Moreover, we correlated differences in the above-mentioned measures observed between High-IQ-22q11DS and ASD with those observed when comparing the entire samples of 22q11DS to ASD.

Results: The High-IQ-22q11DS was significantly different from ASD in several of the above-mentioned metrics, including overall network structure captured by the average difference in connection strength of individual connections ($p < 0.001$), CDI of multiple variables the trajectory of BT bundle 9 and BDA values of multiple variables in both Bundles 8 and 9. See Supplementary Table 2. Moreover the differences in the above-mentioned metrics measured across High-IQ-22q11DS and ASD samples strongly correlated with the differences observed across the entire samples described in the main text, with regards to differences in strength of individual connections ($R = 0.7$, $p < 0.0001$), CDI values (0.75 , $p < 0.0001$), Trajectories of BT-Bundles ($R = 0.9$, $p < 0.0001$) and BDA values of individual variables ($R = 0.5$, $p < 0.0001$). See Supplementary Figure 3.

Discussion: Overall, results of supplementary analysis 3 show that network differences observed across 22q11DS and ASD were not significantly driven by 22q11DS with intellectual disability. Moreover, the High-IQ-22q11DS were on average younger than the entire 22q11DS sample, and not significantly different to the ASD sample. As such these supplementary analyses show that differences in age across 22q11DS and ASD samples were not significantly contributing to network differences described in the main text.

Supplementary Figure 3:



Correlation of network differences measured in when comparing the entire sample of 22q11DS to ASD, plotted in the x axis and the subsample of High-IQ-22q11DS to the same ASD sample.

Panel A: Correlation of differences in strength of individual network connections.

Panel B: Correlation of differences in CDI

Panel C: Correlation of differences in the average trajectory of BT bundles

Panel D: Correlation of differences in BDA

Supplementary Table 1:

	TD-D	22q11DS High IQ	ASD	P- Value TD-D- ASD	P-Value HC- 22q11DS High IQ	P-Value ASD- 22q11DS High IQ
	N=53	N=40	N=60			
Gender (M/F)	27/26	24/16	29/31	p=0.78	p=0.39	p=0.26
Age	18.5+/. 3.7	19 +/. 4.69	17.7+/. 4.9	p=0.37	p=0.55	p=0.21
Mean Number of Assessments	29.4 +/. 8.8	27.45 +/. 11.02	30 +/. 9.4	p=0.73	p=0.34	p=0.21
Number of Consecutive Assessments	21.90 +/. 7.82	20,25 +/. 9.75	22.22 +/. 8.64	p=0.84	p=0.37	p=0.29
Full Scale IQ	111.2 +/. 11.9	71.9 +/. 8.21	107.7 +/. 15.9	p=0.20	p<0.001	P<0.001

Demographics data for Typically Development Discovery Sample (as in the main text), 22Q High IQ (>70), ASD (as in the main text).

Supplementary Table 2:

CDI (22Q - ASD)	CONNECTIVITY DISSIMILARITY INDEX						BDA 22q - ASD																		
							Cluster- Bundle		1		2		3		4		5		6		7		8		9
	Number of Paths						35	54	57	55	33	34	38	37	57										
	22q11.D5 vs ASD	22q11.D5 vsTD-D	TD-D vs ASD	P-value BT	0.45	0.26	0.69	0.05	0.18	0.10	0.10	0.01	0.03												
	SYMPTOMS	T1	T2	T1	T2	T1	T2	SYMPTOMS	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2					
Lack_Relaxation	0.02	0.00	0.11	0.22	0.06	0.01	Lack_Relaxation	0.04	0.12	1.00	0.05	0.17	1.00	0.67	0.79	0.18	0.65	0.92	0.36	1.00	0.32	1.00	0.02	0.11	1.00
Loneliness	0.27	0.32	0.31	0.34	0.10	0.08	Loneliness	1.00	1.00	1.00	0.97	0.97	0.74	1.00	0.87	0.95	0.80	0.02	1.00	0.07	0.96	0.04	0.05	0.07	0.04
Anxiety	0.03	0.01	0.97	0.88	0.03	0.01	Anxiety	0.58	0.58	1.00	0.62	0.66	1.00	0.70	0.84	0.97	0.24	0.97	0.04	1.00	0.52	0.09	0.16	1.00	0.41
Lack_Happiness	0.01	0.09	0.02	0.07	0.28	0.73	Lack_Happiness	0.71	0.45	0.80	0.25	0.55	0.33	0.76	0.07	0.56	0.15	0.36	1.00	1.00	0.51	0.01	0.00	0.41	0.00
Irritation	0.27	0.29	0.13	0.17	0.05	0.11	Irritation	0.95	1.00	1.00	0.46	0.53	1.00	0.60	0.57	0.02	0.65	0.21	0.07	0.03	0.69	0.21	0.50	1.00	0.42
Sensory_Issue	0.02	0.04	0.91	0.80	0.03	0.03	Sensory_Issue	1.00	1.00	1.00	0.97	0.85	1.00	1.00	0.50	0.09	1.00	0.34	0.03	0.64	0.09	0.09	0.54	0.35	0.00
Lack_Excitement	0.08	0.06	0.13	0.06	0.84	0.52	Lack_Excitement	0.72	0.72	0.80	0.45	0.42	0.21	1.00	1.00	1.00	0.28	1.00	1.00	1.00	0.41	1.00	0.00	0.11	1.00
Sadness	0.36	0.32	0.88	0.65	0.10	0.06	Sadness	1.00	1.00	1.00	0.99	0.63	1.00	0.56	0.82	0.84	0.98	0.04	0.82	1.00	0.11	0.00	0.01	0.22	0.26
Lack_Confidence	0.32	0.15	0.89	0.53	0.95	0.88	Lack_Confidence	0.34	0.77	1.00	0.75	0.72	0.17	0.85	0.66	1.00	0.08	0.47	1.00	1.00	0.51	0.58	0.51	1.00	0.70
Feeling_Rejected	0.48	0.61	0.80	0.66	0.27	0.04	Feeling_Rejected	0.02	1.00	1.00	0.88	0.14	1.00	0.25	0.05	0.79	0.86	0.09	0.79	0.48	0.68	0.00	1.00	1.00	0.66
Feeling_Unsafe	0.04	0.06	0.91	0.98	0.01	0.11	Feeling_Unsafe	0.48	1.00	1.00	0.07	0.51	1.00	1.00	1.00	0.94	0.13	0.76	0.35	0.22	0.12	0.62	0.70	1.00	0.20
Confusion	0.29	0.22	0.58	0.80	0.51	0.45	Confusion	0.89	1.00	1.00	1.00	0.89	0.86	1.00	0.27	0.51	1.00	1.00	0.43	0.89	0.43	1.00	1.00	1.00	0.19
Hallucinations	0.08	0.09	0.89	0.97	0.39	0.18	Hallucinations	1.00	1.00	1.00	0.18	0.81	1.00	1.00	0.88	1.00	1.00	0.56	0.20	0.59	0.13	0.05	1.00	1.00	0.32
Feeling_Tired	0.03	0.01	0.00	0.00	0.32	0.31	Feeling_Tired	0.11	0.11	0.09	0.05	0.79	0.63	1.00	1.00	0.78	0.28	1.00	1.00	1.00	0.05	0.39	0.22	0.59	0.23
Lack_Motivation	0.02	0.02	0.02	0.02	0.23	0.85	Lack_Motivation	0.49	0.53	0.02	0.81	0.95	0.82	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.20	0.01	0.26	1.00	1.00
Lack_Physical_Activity	0.05	0.09	0.79	0.75	0.13	0.04	Lack_Physical_Activity	1.00	1.00	0.23	0.47	0.03	0.23	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.04	0.00	1.00	1.00
Finding_Activity_Difficult	0.02	0.03	0.66	0.18	0.10	0.15	Finding_Activity_Difficult	1.00	0.51	0.20	0.62	0.21	0.14	0.17	0.25	0.03	0.46	1.00	1.00	1.00	0.08	0.00	0.67	0.00	0.67
Lack_Enjoying_Activity	0.00	0.02	0.01	0.13	0.22	0.58	Lack_Enjoying_Activity	0.14	0.00	0.66	0.23	0.02	0.12	1.00	0.92	0.66	0.78	0.27	1.00	1.00	0.28	0.45	0.03	0.00	0.66
Lack_Concentration	0.65	0.19	0.11	0.05	0.08	0.17	Lack_Concentration	1.00	0.05	0.96	0.23	0.01	0.08	1.00	1.00	1.00	1.00	0.08	1.00	1.00	0.36	0.17	0.01	0.04	1.00
Being_Alone	0.69	0.35	0.01	0.02	0.04	0.02	Being_Alone	1.00	1.00	1.00	0.35	1.00	0.86	1.00	0.94	0.98	0.84	0.08	1.00	0.74	0.59	0.06	0.04	1.00	0.16

CDI (22Q High IQ - ASD)	CONNECTIVITY DISSIMILARITY INDEX						BDA 22q High IQ - ASD																			
							Cluster- Bundle		1		2		3		4		5		6		7		8		9	
	Number of Paths						35	54	57	55	33	34	38	37	57											
	22q11.D5 High IQ vs ASD	22q11.D5 High IQvsTD-D	TD-D vs ASD	P-value BT	0.45	0.53	0.87	0.12	0.17	0.24	0.31	0.00	0.10													
	SYMPTOMS	T1	T2	T1	T2	T1	T2	SYMPTOMS	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2						
Lack_Relaxation	0.01	0.00	0.98	1.00	0.06	0.01	Lack_Relaxation	0.10	0.34	0.24	0.57	0.10	0.61	0.15	0.22	0.39	0.39	0.25	0.46	1.00	0.09	0.44	0.01	0.64	0.49	
Loneliness	0.07	0.03	0.35	0.11	0.10	0.08	Loneliness	1.00	1.00	1.00	0.59	0.83	0.62	0.29	0.38	0.16	0.25	0.28	0.33	0.61	0.95	0.00	0.22	1.00	0.00	
Anxiety	0.04	0.02	0.72	0.44	0.03	0.01	Anxiety	0.48	0.39	1.00	0.67	0.63	1.00	0.66	0.61	0.66	0.51	0.29	0.83	0.12	0.29	0.03	0.24	0.10	0.71	
Lack_Happiness	0.01	0.03	0.27	0.21	0.28	0.73	Lack_Happiness	0.55	0.05	0.83	0.72	0.23	0.28	0.91	0.27	0.43	0.37	0.03	1.00	1.00	0.25	0.00	0.28	0.77	0.00	
Irritation	0.08	0.10	0.88	0.95	0.05	0.11	Irritation	0.51	0.88	1.00	0.74	0.78	1.00	0.74	0.02	0.88	0.32	0.36	0.23	0.05	0.79	0.13	0.55	1.00	0.25	
Sensory_Issue	0.01	0.06	0.39	0.35	0.03	0.03	Sensory_Issue	1.00	1.00	1.00	1.00	0.67	1.00	0.42	0.44	0.13	0.44	0.90	0.97	0.45	0.46	0.02	0.46	0.46	0.00	
Lack_Excitement	0.21	0.11	0.40	0.41	0.84	0.52	Lack_Excitement	0.76	0.48	0.87	0.65	0.24	0.19	1.00	1.00	1.00	0.46	1.00	1.00	1.00	0.32	1.00	0.06	0.13	0.04	
Sadness	0.00	0.00	0.95	0.92	0.10	0.06	Sadness	1.00	0.02	1.00	0.92	0.51	1.00	0.02	0.00	0.32	0.00	0.01	0.59	0.60	0.87	0.01	0.01	0.44	0.40	
Lack_Confidence	0.07	0.16	0.70	0.86	0.95	0.88	Lack_Confidence	0.15	0.84	1.00	0.57	0.18	0.35	0.10	0.02	0.51	0.00	0.84	0.58	0.34	0.54	0.00	0.41	1.00	0.35	
Feeling_Rejected	0.04	0.02	0.99	0.93	0.27	0.04	Feeling_Rejected	1.00	0.89	1.00	0.58	1.00	1.00	0.37	0.02	0.70	0.38	0.17	0.28	0.73	0.35	0.06	1.00	1.00	0.63	
Feeling_Unsafe	0.01	0.07	0.32	0.89	0.01	0.11	Feeling_Unsafe	0.64	1.00	1.00	0.46	0.23	1.00	1.00	0.43	0.43	0.66	0.29	0.71	0.07	0.77	0.00	0.51	1.00	0.56	
Confusion	0.01	0.01	0.11	0.00	0.51	0.45	Confusion	0.80	1.00	1.00	0.95	0.88	1.00	1.00	0.54	0.51	1.00	1.00	0.49	0.63	1.00	0.07	0.07	1.00	0.15	
Hallucinations	0.01	0.00	0.50	0.64	0.39	0.18	Hallucinations	1.00	1.00	1.00	0.74	0.76	1.00	0.01	0.01	1.00	1.00	1.00	0.50	0.54	0.08	0.04	1.00	1.00	0.32	
Feeling_Tired	0.35	0.21	0.02	0.02	0.32	0.31	Feeling_Tired	0.37	0.49	0.59	0.52	0.82	0.80	1.00	1.00	0.08	0.63	1.00	1.00	1.00	1.13	0.85	0.03	0.00	0.55	0.37
Lack_Motivation	0.00	0.03	0.10	0.00	0.23	0.85	Lack_Motivation	0.45	0.72	0.65	0.42	0.40	0.79	0.06	1.00	0.00	0.00	1.00	1.00	0.80	0.07	0.00	0.29	1.00	1.00	
Lack_Physical_Activity	0.06	0.00	0.25	0.52	0.11	0.04	Lack_Physical_Activity	1.00	1.00	0.17	0.35	0.00	0.25	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.05	0.00	1.00	1.00	
Finding_Activity_Difficult	0.14	0.26	0.72	0.15	0.10	0.15	Finding_Activity_Difficult	1.00	0.54	0.35	0.90	0.65	0.70	0.53	0.43	0.00	0.14	0.01	1.00	1.00	0.02	0.00	0.79	0.00	0.77	
Lack_Enjoying_Activity	0.03	0.11	0.00	0.07	0.22	0.58	Lack_Enjoying_Activity	0.72	0.00	0.74	0.26	0.58	0.44	1.00	0.54	0.43	0.87	0.11	0.05	1.00	0.77	0.15	0.00	0.00	0.75	
Lack_Concentration	0.09	0.16	1.00	1.00	0.08	0.17	Lack_Concentration	1.00	0.13	0.92	1.00	0.17	0.44	1.00	1.00	1.00	1.00	0.83	1.00	1.00	0.02	1.00	0.00	0.11	1.00	
Being_Alone	0.70	0.42	0.00	0.00	0.04	0.02	Being_Alone	1.00	1.00	1.00	0.09	0.09	0.87	0.15	0.45	0.13	0.15	0.00	0.21	0.85	0.85	0.00	0.10	1.00	0.00	

