Network Analysis of Neurotransmitter Related Human Kinase Genes. Possible Role of SRC, RAF1, PTK2B?

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Previous co-expression analysis of human kinase genes highlighted 119 genes in neurotransmitter-related activity (based on Go:Terms). Using a merged interactome dataset, we analyzed the network of these Neurotransmitter Related Human Kinase Genes. Using the full interactome dataset we extended the network and calculating degrees and closeness centralities we identified SRC, MAPK1, RAF1, PTK2B and AKT1 kinase genes as potentially relevant nodes which did not show relevant activity in the original experimental study. As AKT1 and MAPK1 have already been indicated in various neuronal functions, we hypothesize a potential direct or indirect role for SRC, RAF1, PTK2B genes in neurotransmission and in central nervous system signaling processes.

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Kinome stands in the front of current medical research, since kinase enzymes catalyze the transfer of the γ-phosphate from nucleoside triphosphates, carry out the phosphorylation of many proteins, and thus have an essential role in a vast number of biochemical interactions (Cruz & Uckun, 2013). Phosphorylation plays a key role in a wide range of cellular processes (Part et al., 2005).

Kilpinen et al. carried out a detailed co-expression network analysis via studying the levels of 459 human kinase genes in 44 healthy and 55 malignant human tissues (Kilpinen et al., 2010). Using Kilpinen's dataset we filtered for certain neurotransmitter activity related kinase genes based on Go:Terms and analyzed the network of this neurotransmitter-related-kinase genes set in the publicly available merged human interactome (Unilever-PPI) dataset (Garrow et al., 2007). As a second step we added kinase genes and non-kinase genes one step further in the full Unilever-PPI (Garrow et al., 2007) network. In order to localize other key molecules we compared the degrees of the kinase genes of the extended network with the degrees of the full Unilever-PPI network (Garrow et al., 2007). Some kinase genes seemed to become central nodes in the extended network, hence we added these to the original network and analyzed their closeness centrality and used clustering method described by Raghavan et al. (2007).

Based on the results we hypothesize that the c-Src tyrosine kinase (SRC), mitogen-activated protein kinase 1 (MAPK1), RAF proto-oncogene serine/threonine-protein kinase (RAF1), v-akt murine thymoma viral oncogene homolog 1 (AKT1) and protein tyrosine kinase 2 beta (PTK2B) human protein kinase genes might be related to certain neurotransmitter activities. It is widely known that all of these genes are expressed in neurons or glial cells, and they might have an important role in several central nervous system pathologies (Huveldt el al., 2013; Li et al., 2012; Zhang et al., 2013; Lev et al., 1995; Avraham et al., 1995). Thus, based on the described network analysis we hypothesize a possible role of these genes in neurotransmission and in central nervous system signaling processes.

METHOD

Definitions

In the article we used neurotransmitter-related functions as an umbrella term containing the following eight GO terms (The Gene Ontology Consortium,

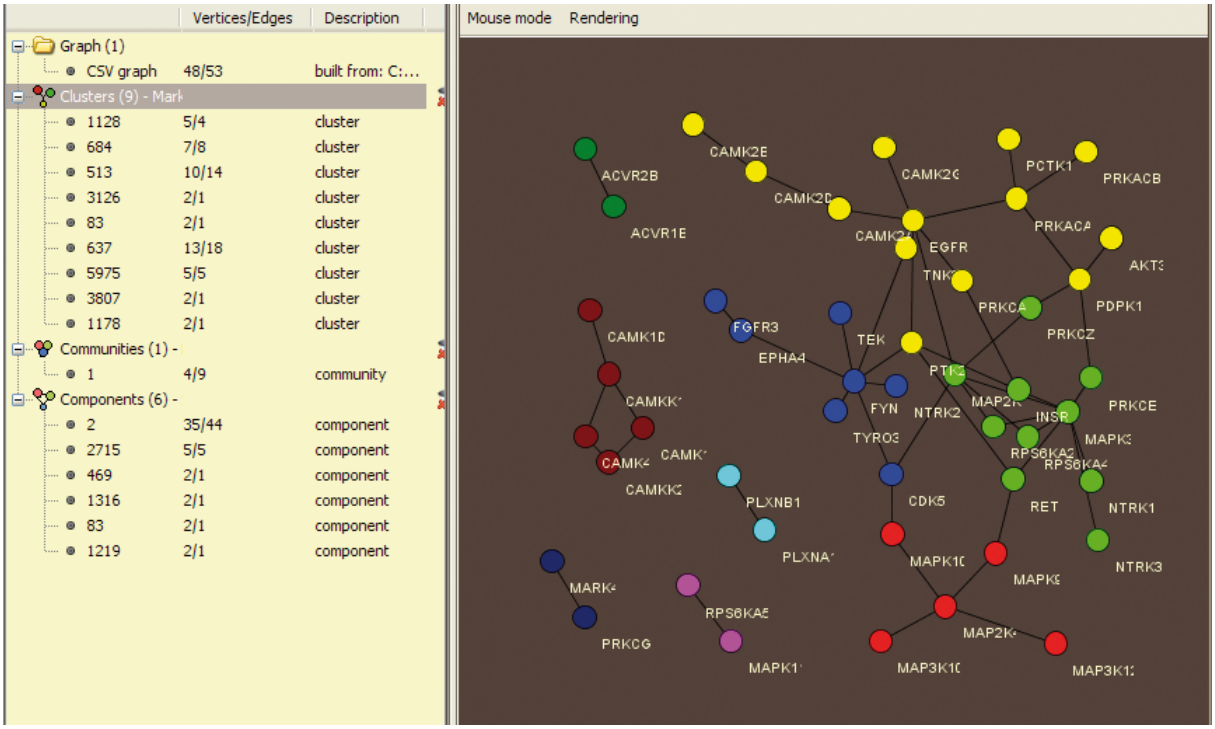

Figure 1 Clusters of the NARKG-1 graph. Nodes are non-single nodes; all are human kinase genes, which seem to be related to neurotransmitter related activity based on the Kilpinen et al. study. Edges represent interaction between two nodes based on Unilever-PPI. Color of the node represents its cluster, following the clustering method described by Raghavan et al (2007). Visualization was done with Sixtep Network Software.

2008): (1) neurotransmitter secretion GO:0007269, (2) neurotransmitter transport GO:0006836, (3) neurotransmitter uptake GO:0001504, (4) positive regulation of neurotransmitter secretion GO:0001956, (5) positive regulation of neurotransmitter transport GO:0051590, (6) regulation of neurotransmitter levels GO:0001505, (7) regulation of neurotransmitter secretion GO:0046928, (8) sequestering of neurotransmitter GO:0042137. Other neurotransmitter related GO:Terms (e.g. GO:0007268, GO:0042165, GO:0010554, GO:0030594, GO:0042133, GO:0042135, GO:0005326, GO:0023005, GO:0042136, GO:0051580, GO:0051609, GO:0071911, GO:0071912, GO:0051588) were exluded as they were not listed among the corresponding GO classes in the Kilpinen et al. study.

In this article we use the set of human kinase genes defined by Park et al (2005): 663 genes, 511 protein kinase, and 152 non-protein kinase. We use the term of Neurotransmitter Related Human Kinase Genes meaning the 119 human kinase genes, which were identified by Kilpinen et al. (2010) in relation to neurotransmitter-related functions.

Analysis

Among the 119 Neurotransmitter Related Human Kinase Genes in the Kilpinen study 42 are in involved in more than one neurotransmitter-related GO Terms, 16 (EPHB6, MAST1, MAP2K1, MAPK11, DCLK1, SBK1, PRKACA, WNK1, CAMK1G, MAP2K4, ACVR2B, CAMK1, PAK7, PAK3, PCTK1) are in more than 2 and one (CDK5) in four neurotransmitter-related GO terms.

For this study we used Unilever-PPI (Garrow et al., 2007) because of its free availability and thoughtful, easy-to-use structure. Unilever-PPI (Garrow et al., 2007) contains 10205 proteins and 58675 interactions (without duplicated edges and self-loops). For the network analysis we used Cytoscape (Shannon et al., 2003) and Sixtep Network Software.

With the exception of 18 genes, all the Neurotransmitter Related Human Kinase Genes were represented at Unilever-PPI (Garrow et al, 2007). As the degree distribution of these 101 genes in Unilever-PPI showed regimes where power law rules network analysis on the subset was supported.

Table 1 Basic network parameters of the NARKG1 and its one step extended graph

	Vertices	Edges	Clustering Coefficient	Components	Diameter	Radius	Density
NARKG1-graph	48	53	0.046	6	8	1	0.047
NARKG1-graph with 1 step extended	1361	8976	0.18	2	8	1	0.033

Table 2 Basic network parameters of the two NARKG-graphs

	Vertices	Edges	Clustering Coefficient	Components	Diameter	Radius	Density
NARKG-1 graph	48	53	0.046	6	8	1	0.047
NARKG-2 graph	157	410	0.174	5	9	1	0.033

Table 3 The first 8 genes with the highest interpretable Closeness Centrality in NARKG-2 graph

Gene symbol	Interpretable Closeness Centralities at NARKG-2 graph
RAF1	0.43274854
SRC	0.43023256
PRKCD	0.41111111
MAPK1	0.39892183
PTK2B	0.39892183
AKT1	0.39572193
LCK	0.39466667
LYN	0.39257294

First we derived a graph using these 101 kinase genes. Isolated nodes, self-loops and duplicated edges were erased from the graph resulting in a graph consisting 48 nodes and 53 edges. In our paper we call this NARKG-1 (Neurotransmitter-Activity-Related-Kinase-Genes-1) graph.

Using Sixtep Network Software of analyzing NARKG-1 graph we identified 6 components and 9 clusters in the network. The biggest component contained 35 kinase genes and 44 interactional connections; center node of the component seemed to be PTK2 gene. This component contained 4 clusters (centered on the following genes: FYN, MAP3K, EGFR, MAP2K4), cluster of FYN and the cluster of MAP3K were more connected than the other two (Figure 1).

Five members of the calcium/calmodulin-dependent protein kinase subfamily formed an independent component and cluster (Figure 1 – brown cluster), in which CAMKK1 seemed to be a center node. Five members of the mitogen-activated protein kinase subfamily also formed a cluster, in which MAP2K4 was in the center. (Figure 1 – red cluster). Members of the FYN-cluster (Figure 1 – blue cluster) are mostly composed of the protein-tyrosine kinase oncogene family.

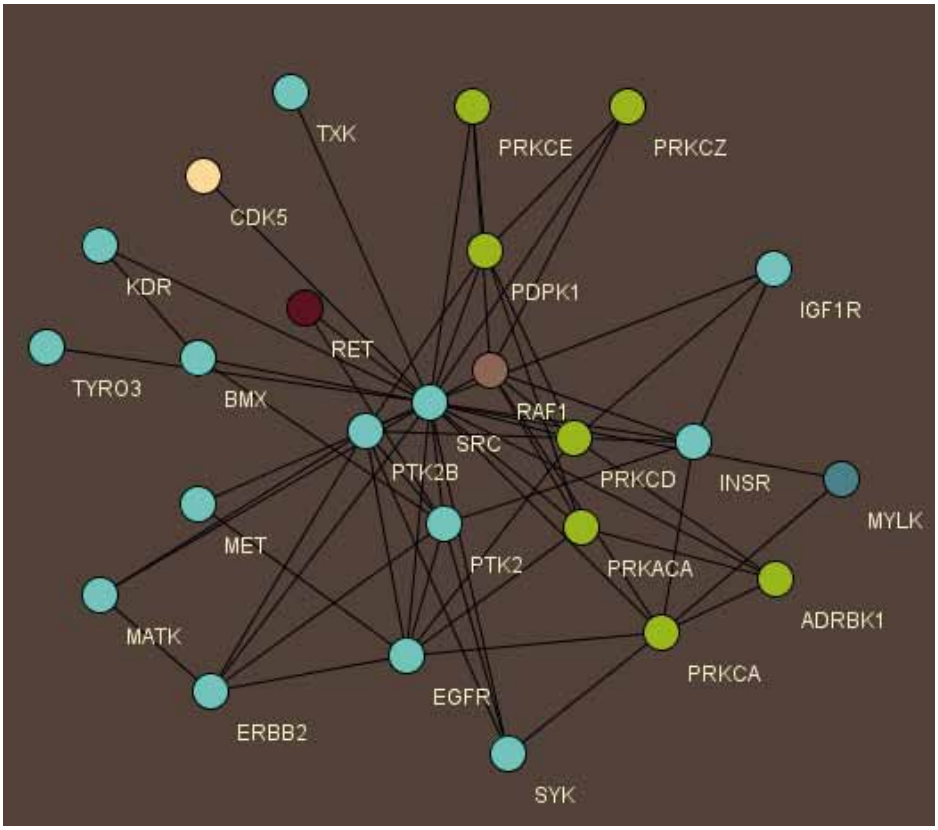

Figure 2 Visualization of a subset of the NARKG-2-graph: 1 depth neighbours of SRC kinase gene. Nodes are non-single nodes; all are human kinase genes. Edges represent interaction between two kinase genes based in the Unilever-PPI data, Color of the node represents its kinase group. Visualization was done with Sixtep Software.

In order to define human kinase genes which might be involved in neurotransmitter-related activity, yet were not exposed in the Kilpinen experimental study, we compared vertex degree distributions of the NARK1 one step extended graph with the full Unilever-PPI network. Self-loops, duplicated edges, and one isolated node were erased from the graph. Density of the extended network was lower and the clustering coefficient was significantly higher. (Table 1)

According to our hypothesis in the extended graph non-neurotransmitter-related kinase genes shall be less represented (proportionally to the number of the edges) and neurotransmitter-related-kinase-genes shall be represented more.

We analyzed proportional degree distribution of the extended graph and found that the 75 neurotransmitter-related-kinase-genes were proportionally being represented higher. No neurotransmitter related kinase genes were found among those human kinase genes which were represented proportionally less in the extended graph. Both results supported our hypothesis.

However, according to our theoretical model, those humane kinase genes, which were intensively represented in the extended network but were not listed among the Neurotransmitter Related Human Kinase Genes by the Kilpinen study might still play a role in neurotransmitter-related activity. To theoretically examine our hypothesis we added these 96 genes to the existing 101 and created a subset of the Unilever-PPI dataset (Garrow et al., 2007). After removing single nodes (39) a graph containing 157 human kinase genes and 410 interactions among them were derived, we call this Neurotransmitter-Activity-Related-Kinase-Genes-2 (NARKG-2) graph.

As the clustering coefficient of NARKG-2 was higher and yet the density was lower than NARKG-1 graph, it suggested that the newly added kinase genes had "embedded" into the NARKG-1 graph. (Table 2 shows the basic parameter of NARKG-1 graph and

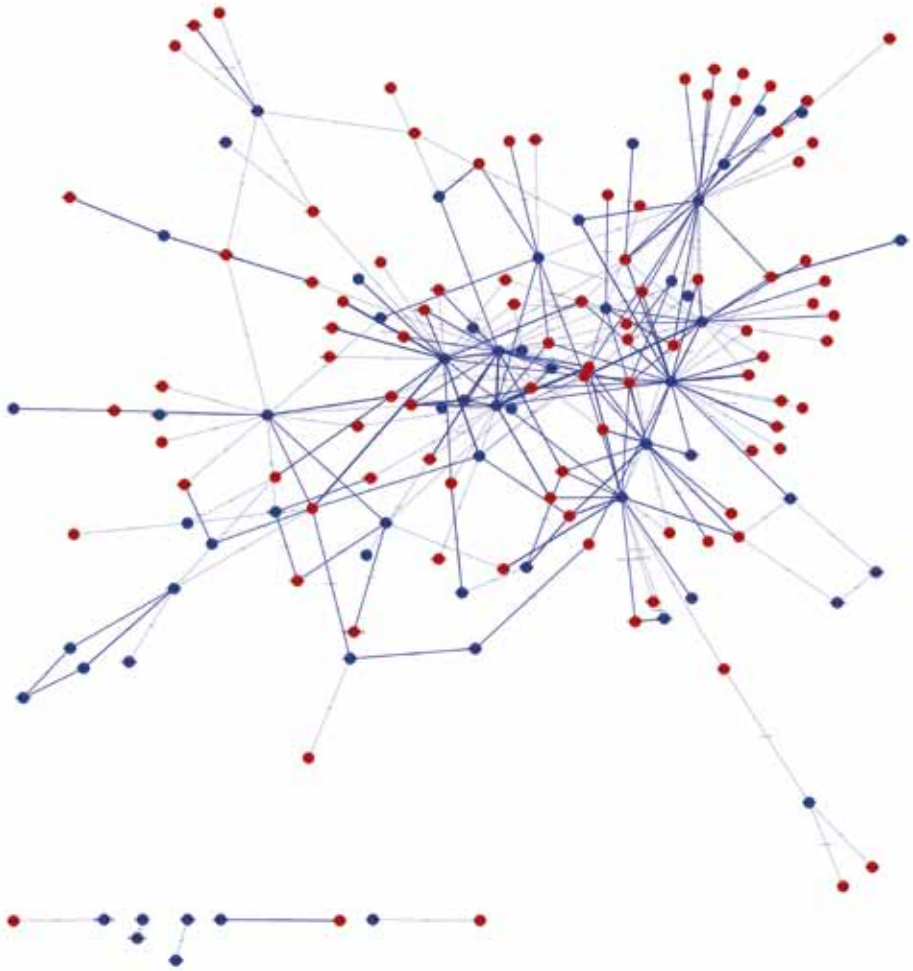

Figure 3 Visualization of NARKG-2 graph without edges connecting only non-NARKG-1 nodes. All nodes are non-single nodes; all are human kinase genes. Edges represent interaction between two kinase genes based in the Unilever-PPI data; width of the edge represents the weight of the interaction. Edges are labeled. Color of the node represent whether it is an added node (red) or NARKG-1 node (blue). Visualization was done by Cytoscape.

NARK2 –graph compared, Figure 2 visualize a subset of the NARK2-graph.)

We have also analyzed the clusters of NARK2-graph using Sixtep Network Software. We identified 4 components, 13 clusters (following the Raghavan et al. work) and 6 communities (containing more than 10 vertexes). Similar kinase group members tend to cluster; the biggest cluster was mostly composed out of TK Kinase Group members. The second largest cluster was mostly composed out of genes belonging to the AGC Kinase Group.

The degree distribution of the added kinase genes followed power of law distribution in NARKG-2 graph; we anticipated that the potential significance of the added kinase genes (in neurotransmitter-related activity) probably corresponds to higher vertex degrees. High-degree vertexes (genes) seemed to result in well-researched kinase genes such as SRC, MAPK1, RAF1, PTK2B, AKT1, etc. Analysis of the Closeness Centrality, which in many cases means functional relevancy for other proteins (Chea & Livesay, 2007) also confirmed the potential functional relevancy of RAF1, SRC, PTK2B genes. (Table 3)

Analysis of the degree distribution of the newly added nodes, after removing edges connecting only newly added nodes also resulted in the confirmation of the potential functional importance of SRC, MAPK1, RAF1, PTK2B and AKT1 genes. (Figure 3)

Discussion

The SRC, MAPK1, RAF1, PTK2B, AKT1 genes are involved in forming of several types of cancer. The activation of SRC pathway was discovered in liver, lung, breast, colon, and pancreas cancer (Dehm & Bonham, 2004). The c-SRC tyrosine kinase inhibitors have been approved for the treatment of chronic myeloid leukemia and acute lymphocytic leukemia (Breccia et al., 2013).

The AKT1 inhibitors are investigated in trials for the treatment of neuroblastoma, different solid tumors and have been already approved for the treatment of Leismaniasis (Sundar & Olliaro, 2007). The role of AKT1 is also studied in HIV, HSV infections (Cheshenko et al., 2013). AKT1 has been implicated schizophrenia (Chen et al., 2013) and also in Proteus syndrome (Marjorie et al., 2011). AKT1 and MAPK1 both have been implicated in and Alzheimer's disease (Shibata et al., 2011, Kálmán et al., 2012).

As among the five identified kinase genes (SRC, MAPK1, RAF1, PTK2B, AKT1) which might play a direct or indirect role in neurotransmitter-related activity two genes (AKT1, MAPK1) were implicated in neuronal functions, we hypothesize a potential direct or indirect role for SRC, RAF1, PTK2B genes in neurotransmission and in central nervous system signaling processes.

There are three limitations of this study: (1) analyses are based on two databases (Kilpinen et al, 2010, Garrow et al., 2007) and the merged Unilever-PPI (Garrow et al., 2007) is relatively old and does not contain many newly discovered interactions among kinase genes, yet we propose that it catches the general trends; (2) the selected GO:Terms to the neurotransmitter-related functions might be broadened with some other closely related terms, yet we choose it to keep narrow to avoid overbroadening; (3) our current network analysis may only indicate that the SRC, RAF1, PTK2B genes are might be involved in a non-neuronal specific cellular processes, but another profound biochemical way in the process.

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Neurotranszmitterekhez köthető humán kináz gének hálózatos vizsgálata. Az SRC, RAF1, PTK2B lehetséges szerepe?

Korábbi kutatásokban a humán kináz gének ko-expressziós vizsgálata során 119 neurotranszmiter tevékenységhez köthető gént mutattak ki (GO:Term alapján). Egyesített fehérje-fehérje hálózat adatainak felhasználásával a neurotranszmiterekhez köthető kináz gének hálózatát vizsgálatuk. A teljes interaktom adathalmaz segítségével kiegészítettük az alaphálózatot, és fokszám valamint közelség központiság számolásával az SRC, MAPK1, RAF1, PTK2B, AKT1 géneket azonosítottuk, mint esetlegesen releváns (de az eredeti tanulmányban nem kimutatott) csomópontokat. Mivel az AKT1 és MAPK1 több neurológiai funkcióban érintett, vizsgálatunk alapján felvethető, hogy az SRC, RAF1, PTK2B gének is direkt vagy indirekt módon szerepet játszhatnak a neurotranszmitterek, illetve a központi idegrendszer jelátviteli folyamataiban.

Kulcsszavak: hálózati analízis, kináz gén, kinom, bioinformatika, neurotranszmitter