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Network analysis of Urocortins

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Abstract

OBJECTIVES: Urocortins (Ucns), members of corticotropin releasing factor family, play critical roles in a number of pathological and physiological conditions. Many proteins have been reported to participate in Ucns signaling pathways, which formed complex interaction networks.
METHODS: STITCH ('search tool for interactions of chemicals') is an interaction network database that provides exploration of the known and predicted interactions among large sets of chemicals and proteins.
RESULTS: In this study, using STITCH, interaction networks of Ucns were constructed by database mining, and then their topological parameters and important nodes were analyzed by network related tools. This may help a quick and thorough overview of the Ucns mechanisms underlying in a visual format.

INTRODUCTION

Urocortins (Ucns), peptides that belong to the corticotropin-releasing factor (CRF, also known as the corticotropin-releasing hormone, CRH) family found in bony fish, amphibians, birds, and mammals, have unique phylogenies, pharmacologies, and tissue distributions (Fekete & Zorrilla 2007; Garg & Frishman 2013). The 3 types of UCNs (1, 2, and 3) may be clinically relevant molecules in the pathogenesis, treatment or management of many conditions, including congestive heart failure, hypertension, inflammatory disorder (irritable bowel syndrome, active gastritis, and rheumatoid arthritis), atopic/allergic disorders (dermatitis, urticaria, and asthma), gastroparesis, pregnancy and parturition, major depression, and obesity (Fekete & Zorrilla 2007). The past decade witnessed an increasing knowledge on the peripheral expression and regulation of CRF and urocortin signaling systems and recognition of their implication in health and disease (Stengel & Tache 2014). But how to convert information from massive data sets into insight is still a critical challenge (Shneiderman 2014).

Biological network integration, visualization and analysis is a powerful approach to gain systems-level understanding of patterns of gene expression and protein-protein interaction in different cell types, disease states and other biological/experimental conditions (Furlong 2013; Kwoh & Ng 2007; Xia *et al.* 2014). For example, networks for adipoenctin associated target based proteins had been constructed and their topological properties were analyzed (Chen 2013). It was used for analysis of mRNA expression profile of Ezrin knockdown in Esophageal squamous cell carcinoma (Wu *et al.* 2014b). Biological networks have been developed as a platform for integrating information from high- to low-throughput experiments for analysis of biological systems (Kwoh & Ng 2007). Therefore, it will be a powerful tool to gain a systematic view of Ucns.

In this study, the Ucns interaction networks were constructed by database mining. Furthermore, these networks' properties were analyzed by

ORIGINAL ARTICLE

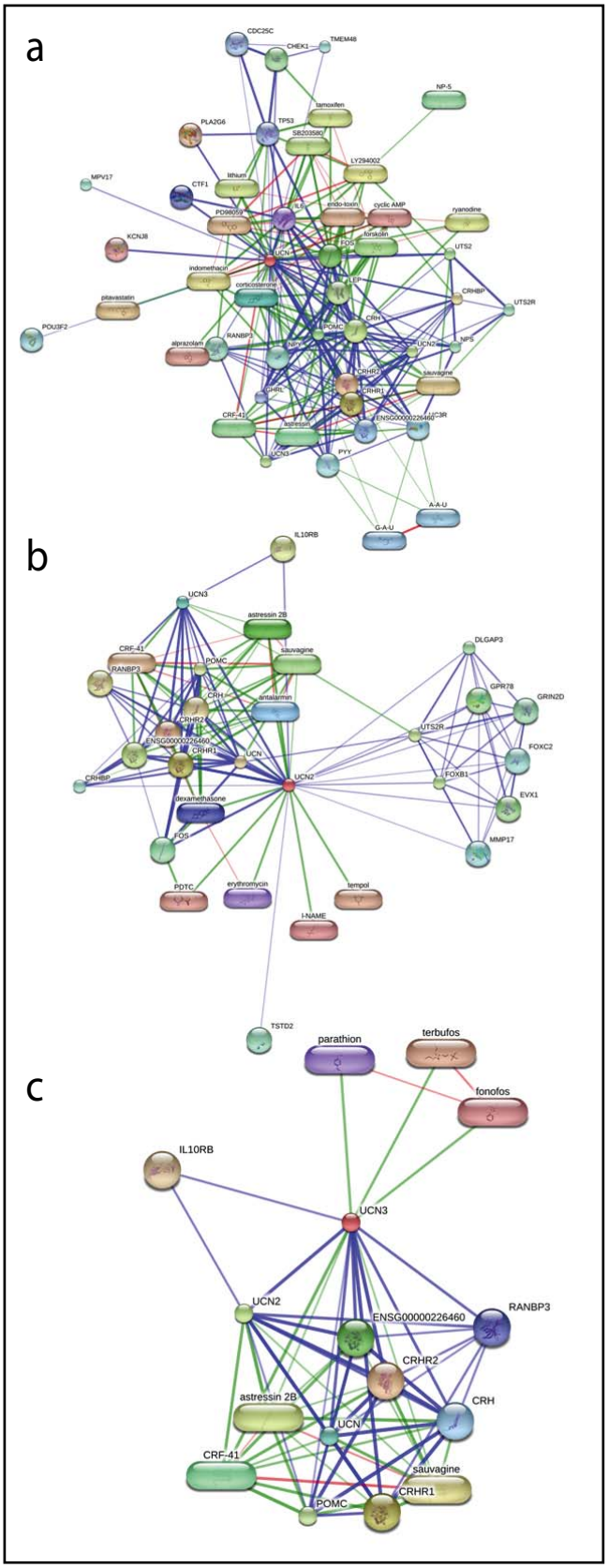

Fig. 1. Ucns interaction networks constructed by STITCH: (A) Ucn1; (B) Ucn2; (C) Ucn3. Nodes: specific proteins or chemical compounds; edges: interactions. Red spheres in the central: Ucns; spheres of other: interacting proteins; oblong shapes: chemicals. Protein-protein interaction: blue lines; chemical-protein interaction: green lines. Stronger associations are represented by thicker lines.

computational tools. The purpose of this study was to facilitate a system-level understanding of Ucns interactions in a visual pattern.

MATERIALS AND METHODS

Network construction via STITICH

STITCH (available at http://stitch.embl.de) is a database of protein-chemical interactions that integrates sources of experimental and manually curated evidence with text-mining information and interaction predictions (Kuhn *et al.* 2008; Kuhn *et al.* 2010; Kuhn *et al.* 2012; Kuhn *et al.* 2014a). The resulting interaction network includes 390 000 chemicals and 3.6 million proteins from 1133 organisms (Kuhn *et al.* 2014a). Recently, in a study, functional chemical-protein association analysis was performed to retrieve multi-target drugs of high pathway wideness from the STITCH 3.1 database (Xu *et al.* 2014). In a research on psoriasis, SITITCH was used for chemical-protein interaction network construction (Manczinger & Kemeny 2013). Moreover, querying STITCH for a protein will provide the user with a network that places the protein into its chemical and biological context (Kuhn *et al.* 2014a; Kuhn *et al.* 2012; Kuhn *et al.* 2010; Kuhn *et al.* 2008).

CRH, Urocortin1, Urocortin2, Urocortin3 (Homo sapiens) was entered into the STITCH 4.0 search panel respectively. The medium confidence of the required confidence (score) was set to 0.400. The maximum number of interactions was set to 500. The other parameters were kept as default values. Then, the constructed networks were exported.

Topological parameters analyzed by NetworkAnalyzer

Cytoscape is an established free open-source software platform for the visualization and analysis of molecular interaction networks (Shannon *et al.* 2003; Saito *et al.* 2012). It can be extended through plugins, enabling a broad community of scientists to contribute useful features (Saito *et al.* 2012). NetworkAnalyzer plugin is installed in Cytoscape by default and computers and displays a comprehensive set of topological parameters such as the number of nodes, edges, and connected components, the network diameters, radius, density, centralization, heterogeneity and so on (Saito *et al.* 2012; Assenov *et al.* 2008). The plugin was used in a protein-protein interaction network in coronary artery disease (Nair *et al.* 2014), and in a research on esophageal squamous cell carcinoma (Wu *et al.* 2014a).

The networks obtained in the previous step were imported into NetworkAnalyzer and treated as undirected. The other parameters were set to default values.

Important nodes identified by STITCH

STITCH assigns a confidence score for each chemical–protein and chemical–chemical interaction (Li *et al.* 2013; Kuhn *et al.* 2010; Kuhn *et al.* 2012; Kuhn *et al.* 2014b; Kuhn *et al.* 2008). For this analysis, CRH, Uro-

cortin1, Urocortin2, and Urocortin3 (*Homo sapiens*) were entered into the STITCH search panel individually, and the parameter settings were set as described above.

RESULTS

Network constructed by STITCH 4.0

The interaction networks of Ucns were constructed and downloaded from STITCH 4.0 by database mining on May 26, 2014. As shown in Figure 1 (Ucn1, 1a; Ucn2, 1b; Ucn3, 1c), the Ucn1 network has 49 nodes (proteins or chemical compound) and 239 edges (interactions), the Ucn2 network is made up of 30 nodes and 132 edges, and Ucn3 consists of 16 nodes and 65 edges (Table 1). Meanwhile, the CRH network has 281 nodes and 9174 edges (supplementary Table 1).

Topological parameters analyzed by NetworkAnalyzer

As shown in Figure 2 and listed in Table 1, the topological properties of the Ucns networks are accessible. The degree distributions, average clustering coefficients, topological coefficients, and characteristic path lengths are visualized in Figure 2. Other parameters such as network diameters, average number of neighbors, network density, clustering coefficient, and so forth, are also shown in Table 2.

Top 10 nodes identified by STITCH

According to their confidence scores, STITCH 4.0 identified the 10 top nodes in each network (Table 2). The top 2 nodes in all three networks are corticotropin releasing hormone receptor (CRHR1) and CRHR2.

Tab. 1. Detailed topological parameters of Ucns networks analyzed by NetworkAnalyzer.

Parameter	UCN1	UCN2	UCN3
Number of nodes	49	30	16
Number of edges	239	132	65
Characteristic path length	1.836	1.697	1.458
Network diameter	3	2	2
Avg. number of neighbors	9.510	8.8	8.125
Network density	0.198	0.303	0.542
Clustering coefficient	0.614	0.730	0.855
Network heterogeneity	0.853	0.623	0.464

Tab. 2. The 10 top nodes in each Ucns network according to the confidence scores by STITCH.

Rank	UCN1	UCN2	UCN3
1	CRHR2	CRHR2	CRHR2
2	CRHR1	CRHR1	CRHR1
3	CRH	ENSG00000226460	UCN2
4	FOS	Astressin 2B	ENSG00000226460
5	astressin	FOS	CRF-41
6	corticosterone	UCN3	UCN
7	ENSG00000226460	Antalarmin	CRH
8	CTF1	Dexamethasone	RANBP3
9	IL6	Erythromycin	Parathion
10	KCNJ8	I-NAME	fonofos

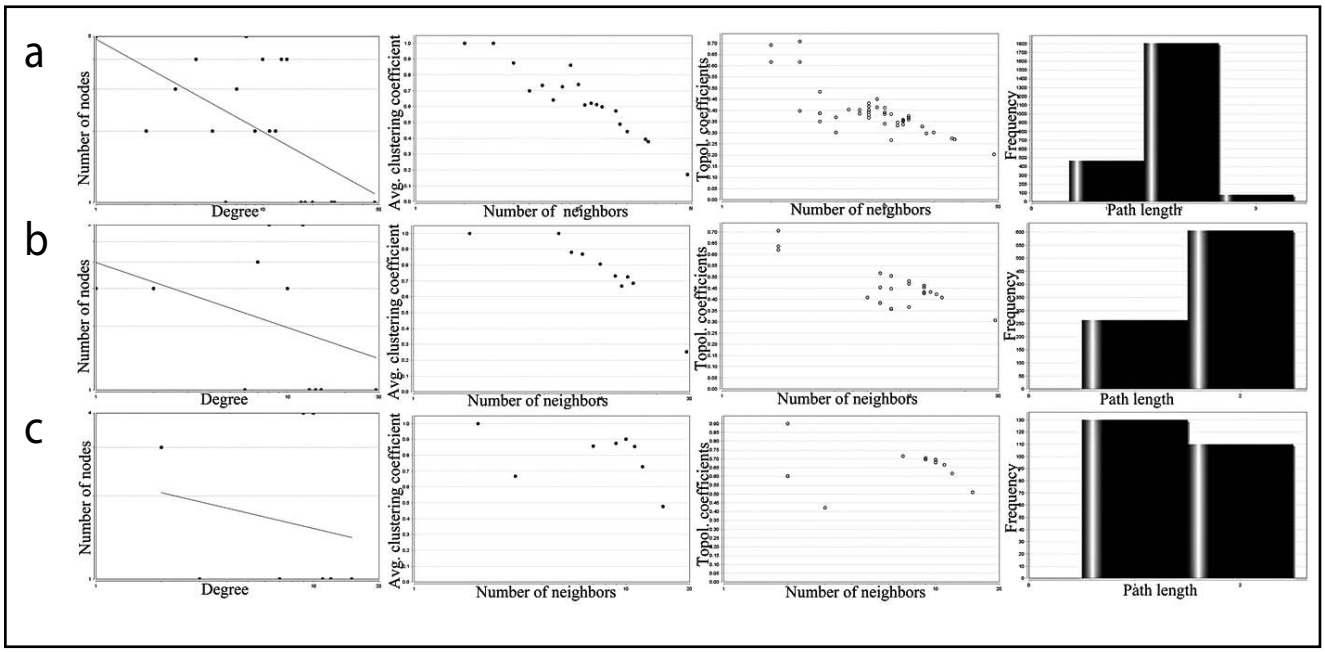

Fig. 2. Schematic diagram of topological parameters for Ucns networks, showing the number/degree of nodes, the average clustering coefficient/number of neighbors, the topology coefficient/number of neighbors, and the frequency/path length: (A) Ucn1; (B) Ucn2; (C) Ucn3.

DISCUSSION

The biological system is a complex physicochemical system consisting of numerous dynamic networks of biochemical reactions and signaling interaction between cellular components, which makes it virtually unanalyzable by traditional methods (Kwoh & Ng 2007). Therefore, construction and analysis of biological networks is vital for successful quantitative modeling of biological systems (Kwoh & Ng 2007). Shown in Figure 1, the interaction network will help us a system-level understanding of complex biological activities of Ucns.

Global network properties are determined to assess the overall characteristics of the network such as how they are formed, what model they fit, how robust they are, and how tightly the elements are connected (Altaf-Ul-Amin *et al.* 2014). No typical nodes existing in all three Ucns networks (Figure 1) and the degree distribution decreasing according to a power law (Figure 2) mean that these networks are scale-free. These results are consistent with the "universal laws" that cellular networks are scale-free (Lima-Mendez & van Helden 2009; Barabasi & Oltvai 2004). Another topological property, the characteristic path length means the expected distance between two connected nodes and the network diameter is the largest distance between two nodes, and the average number of neighbors means the mean number of connection of each node (Bernabo *et al.* 2013; Barabasi & Oltvai 2004; Chen 2013). As shown in Table 1, the Ucn1 network has the longest characteristic path length, the largest network diameter, and the highest average number of neighbors, while Ucn3 networks is in another opposite end. Network density indicates how densely the network is populated with edges (Chen 2013). The value in Ucn3 is 0.542, much higher than those in both ucn1 and ucn2 networks. The average clustering coefficient is the average of the clustering coefficients for all the proteins that form clusters in the network(Chen 2013). The value in Ucn1 network is 0.614, much lower than those of the other two networks, meaning a large number of protein interactions. These parameters are shown in Figure 2 and listed in Table 1. These properties will contribute to a systematic understanding of Ucns networks.

Highly connected hub nodes, central to the network architecture, have been found to play important roles in many networks (Langfelder *et al.* 2013). As listed in Table 2, top 10 nodes in each network were identified. As we known, in addition to CRF, CRF system includes the three Urocortin peptide (Ucn1, Ucn2 and Ucn3), two receptors type (CRFR1 and CRFR2) and CRF-binding protein (Ryabinin *et al.* 2012; Pan & Kastin 2008). Ucns bind and activate the CRFR2 with high affinity, Ucn1 has equal affinities for both receptors; and Ucn2 and 3 appear to be selective for CRFR2 (Ryabinin *et al.* 2012). As expected, in all three networks, both CRFR1 and CRFR2 are the most important nodes respectively (Table 2). Other elements in CRF system, CRFR1 precursor (ENSG00000226460), astressin, dexamethasone, antalarmin also play important roles in the three networks (Table 2). Notably, Fos has important effects in Ucn 1 and Ucn 2 networks (Table 2). The immediate early gene product Fos is part of the activator protein-1 (AP-1) transcription factor and has been shown to participate in molecular mechanisms of cell proliferation, differentiation, apoptosis, and transformation (Durchdewald *et al.* 2009). Ucn 1- induced increase of c-fos mRNA levels in the caudal brain stem containing the nucleus of the solitary tract was inhibited by CRF2 antagonists (Yakabi *et al.* 2011). Injections of Ucn1 into the basolateral amygdala induce anxiety-like and c-Fos expression in brainstem serotonergic neurons (Spiga *et al.* 2006). Ucn 2 increases c-Fos expression in serotonergic neurons projecting to the ventricular/periventricular system (Hale *et al.* 2010), in topographically organized subpopulations of serotonergic neurons in the rat dorsal raphe nucleus (Staub *et al.* 2005). All these suggest that Fos play critical roles in the Ucns networks.

In addition, CRH is the first discovered and the most widely studies peptide in CRH family (Vale *et al*, 1981), it's network is more huge and complex than the three Ucns networks, and can't provide a quick and concise view (supplementary figure 1). So, comparison with UCN networks will be meaningless. But the topological parameters (supplementary table 1) and the top functional partners (supplementary table 2) will also provide some important information to understand the CRH functions.

CONCLUSION

Biology has recently become a "big-data science" mainly supported by the advances in experimental technologies (Altaf-Ul-Amin *et al.* 2014). Network construction and analysis facilitates the system-level understanding of the cell or cellular components and subprocesses (Altaf-Ul-Amin *et al.* 2014). In this article, Ucns interaction networks were constructed via database mining. And the topological parameters and hubs of these networks were analyzed by network-related tools. However, it is noteworthy that two deficiencies exist in this research. One is that all data were retrieved only from STITCH database, the other is that false-positive results may come from data mining. Nevertheless, this study offered a systematic overview of Ucns interaction networks in a compact and visually manner.

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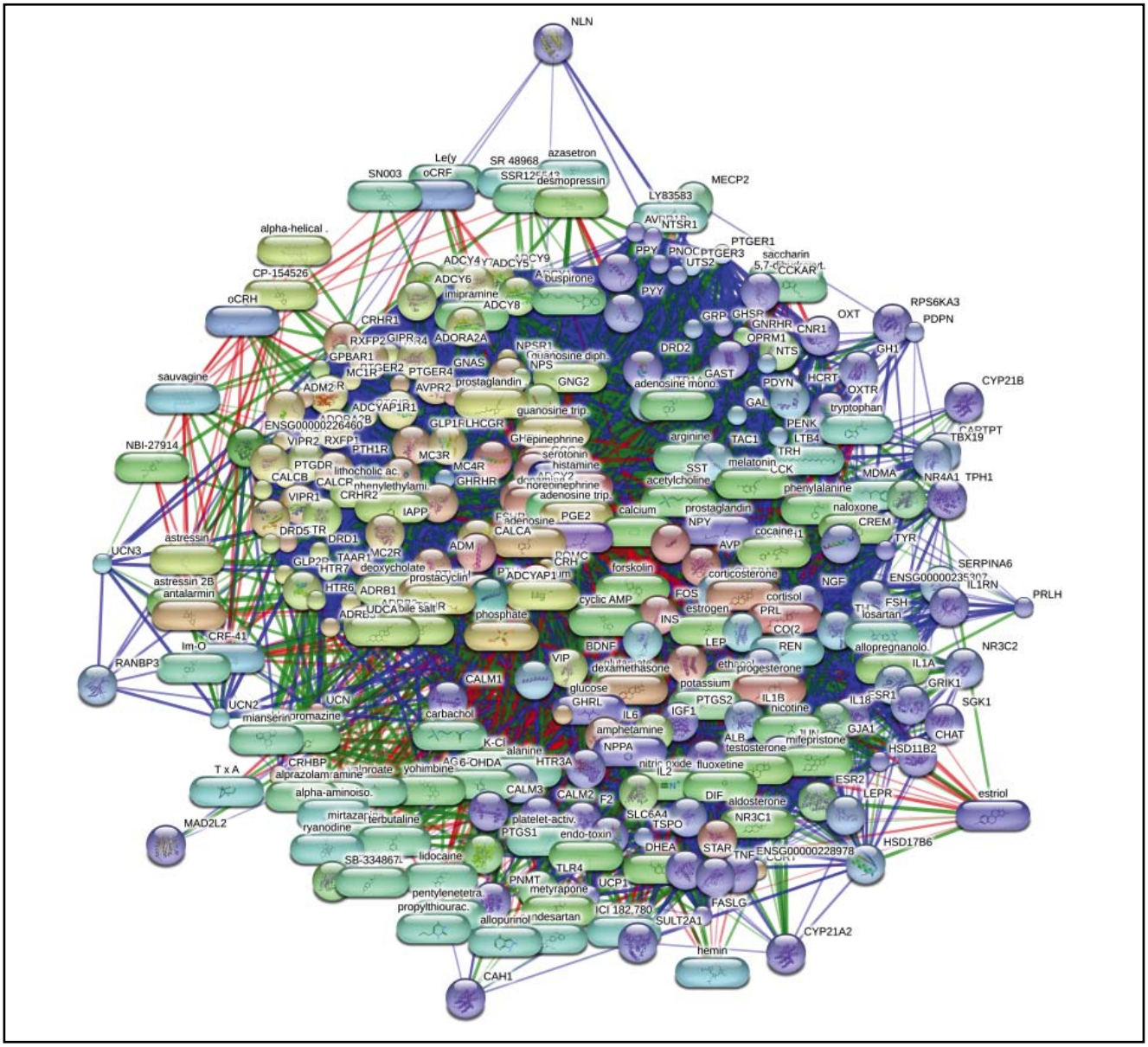

Supplementary Fig. 1. The confidence view of CRH network. Note: spheres shapes: proteins; oblong shapes: chemicals; protein-protein interaction are shown in blue, chemical-protein interaction in green and interactions between chemicals in red.

Supplementary Tab. 1. Detailed topological parameters of CRH network analyzed by NetworkAnalyzer.

Parameter	CRH
Number of nodes	281
Number of edges	9174
Characteristic path length	1.818
Network diameter	4
Avg. number of neighbors	62.235
Network density	0.222
Clustering coefficient	0.658
Network heterogeneity	0.708

Supplementary Tab. 2. The top 10 predicted functional partners in CRH network.

Rank	CRH
1	CRHR1
2	POMC
3	CRHR2
4	ENSG00000226460
5	norepinephrine
6	ADCYAP1
7	MC4R
8	serotonin
9	PGE2
10	histamine