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Identification of Key Proteins Involved in Axon Guidance Related Disorders: A Systems Biology Approach

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Abstract

Axon guidance is a crucial process for growth of the central and peripheral nervous systems. In this study, 3 axon guidance related disorders, namely- Duane Retraction Syndrome (DRS) , Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS) and Congenital fibrosis of the extraocular muscles type 3 (CFEOM3) were studied using various Systems Biology tools to identify the genes and proteins involved with them to get a better idea about the underlying molecular mechanisms including the regulatory mechanisms. Based on the analyses carried out, 7 significant modules have been identified from the PPI network. Five pathways/processes have been found to be significantly associated with DRS, HGPPS and CFEOM3 associated genes. From the PPI network, 3 have been identified as hub proteins- DRD2, UBC and CUL3.

Keywords

Axon Guidance ; DRS ; HGPPS ; CFEOM3 ; *in silico* ; Systems Biology ; PPI network

Introduction

Duane Retraction Syndrome (DRS) is a human genetic disorder of cranial nerve guidance (Engle 2010). It's a disorder of ocular motility involving deficient horizontal eye movements, eyelid retraction, palpebral fissure narrowing in adduction and a variety of other abnormal movement of the affected eye when the other eye is fixated in various cardinal positions (SHALABY and BAHGAT 2010; ALEXANDRAKIS and SAUNDERS 2001) . The absence of 6th cranial nerve and the consequent developmental adaptation that occurs in the embryo results in the abnormal pattern of ocular motility of DRS (SHALABY and BAHGAT 2010).

Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS) is a clinically and genetically homogeneous disorder which is associated with failure of hindbrain axons to cross the midline

(Engle 2010). Symptoms include being born with restricted horizontal gaze and development of scoliosis within the first decade of life. It results from mutations in the ROBO3 gene and is autosomal recessive in nature (Jen et al. 2004).

Congenital fibrosis of the extraocular muscles type 3 (CFEOM3), an autosomal dominant congenital eye movement disorder involves variable unilateral or bilateral ophthalmoplegia, limited vertical ductions, and blepharoptosis (drooping eyelids) (Mackey et al. 2002).

The purpose of this study was to identify crucial genes and proteins associated with Duane Retraction Syndrome (DRS), Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS) and Congenital fibrosis of the extraocular muscles type 3 (CFEOM3) to get a better idea about the molecular mechanism, pathways and the key players. In order to do this, DRS, HGPPS and CFEOM3 associated genes from several databases were retrieved. Various Systems Biology tools have been put to use to carry out several analyses such as pathway enrichment analysis, protein-protein interaction (PPI) network, module analysis and transcriptional regulatory network analysis.

Methods

DRS, HGPPS and CFEOM3 associated genes

The keywords “Duane Retraction Syndrome”, “HGPS”, and “Congenital fibrosis of the extraocular muscles type 3” were used to screen out the genes associated with them from 3 databases and the results were put together to build a set of DRS, HGPPS and CFEOM3 associated genes. The three databases used for finding the genes were-

- GeneCards (version 3.0), a searchable, integrative database which provides comprehensive information on all annotated and predicted human genes (Safran et al. 2010).
- Search Tool for Interacting Chemicals (STICH, version 5.0), a database of known and predicted interactions between chemicals and proteins (Szklarczyk et al. 2016)
- Comparative Toxicogenomics Database (CTD), which provides manually curated information concerning chemical–gene/protein interactions, chemical–disease as well as gene–disease relationships was used (Davis et al. 2017).

PPI Network

Data from 2 interaction databases were merged in order to predict the Protein-Protein Interaction (PPI) pairs among the DRS, HGPPS and CFEOM3 associated genes. The databases were-

- Biological General Repository for Interaction Datasets (BioGRID, version 3.4, https://wiki.thebiogrid.org/) (Chatr-aryamontri et al. 2015)
- The Molecular Interaction Database (MINT, 2012 update, https://mint.bio.uniroma2.it/) (Licata et al. 2012)

Using the software Cytoscape (http://www.cytoscape.org) (Saito et al. 2012), a PPI network was visualized for DRS, HGPPS and CFEOM3 associated genes.

Using the CytoNCA plug-in (Li et al. 2017) (version 2.1.6, http://apps.cytoscape.org/apps/cytonca) in Cytoscape, degree centrality (DC), betweenness centrality (BC), and closeness centrality of the nodes of the PPI network were subjected to analysis to identify the hub proteins (He and Zhang n.d.). “Without weight.” was set as the parameter.

Module Analysis

For module analysis of the PPI network, MCODE plug-in (Bader and Hogue 2003) (version 1.4.2; http://apps.cytoscape.org/apps/mcode; parameters set as degree cut-off = 2, maximum depth = 100, node score cut-off = 0.2, and -core = 2) in Cytoscape was used.

Pathway Enrichment Analysis

KEGG pathway enrichment analysis for the nodes of top modules was carried out with JEPETTO plug-in (Winterhalter, Widera, and Krasnogor 2014) in Cytoscape.

Transcriptional Regulatory Network Construction

Transcription factors (TFs) among DRS, HGPPS and CFEOM3 associated genes were searched and then their targets were identified using the transcriptional regulatory relationships unravelled by a sentence-based text-mining (TRRUST, http://www.grnpedia.org/trrust/) (Han et al. 2015) database.

Finally, a transcriptional regulatory network of the hub proteins was constructed using Cytoscape (Saito et al. 2012).

Results and Discussion

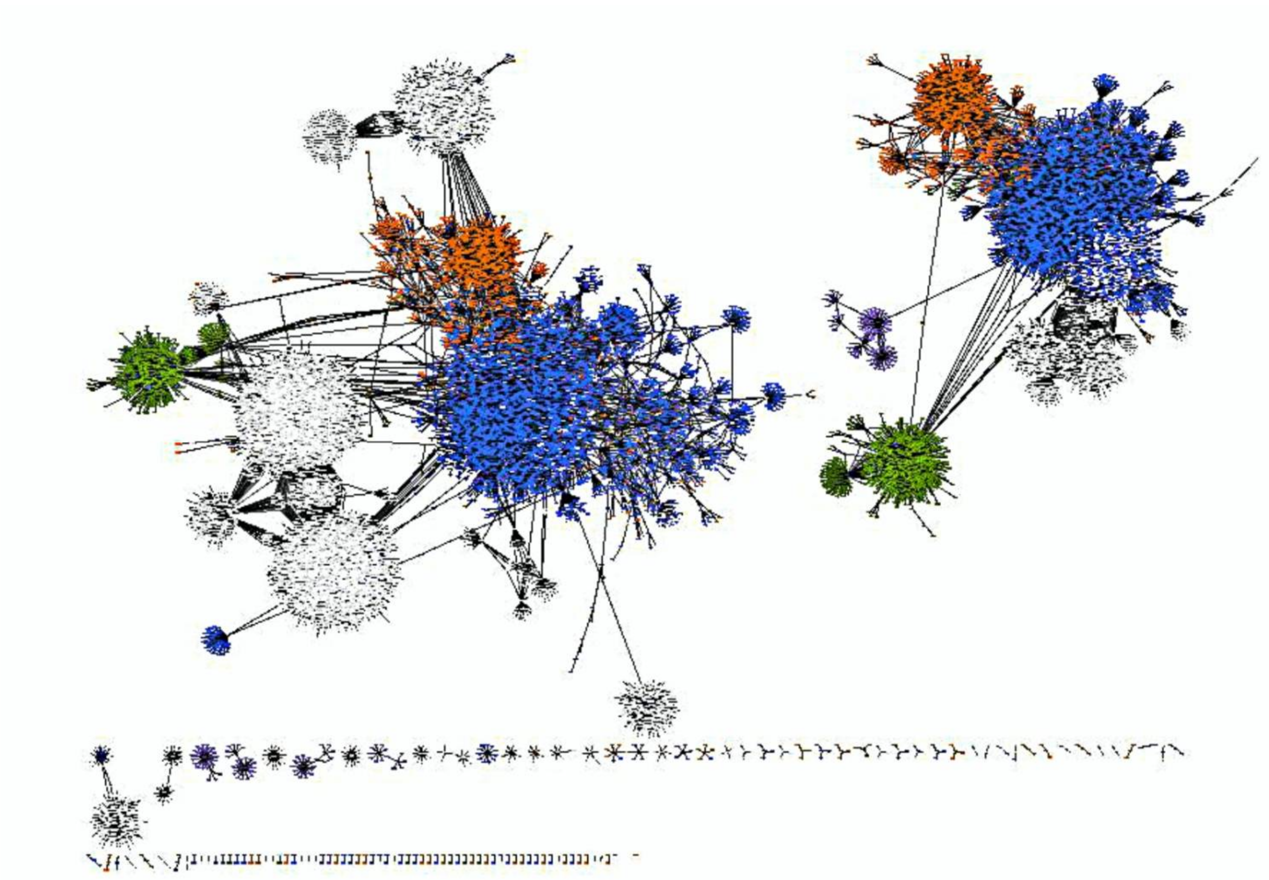

Figure 1: Protein-protein interaction network of with DRS, HGPPS and CFEOM3 associated genes. It contains 24,062 nodes (proteins) and 29,226 edges (interactions).

Table 1: Axon guidance related disorder associated genes

Duane Retraction Syndrome (DRS)	Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS)	Congenital fibrosis of the extraocular muscle type3 (CFEOM3)
CHN1, MAFB, SALL4, SALL1, PAX2, TBX5, CHD7, KIF21A, BMP4, TBX3, TUBB3, ADNP, DPM1, HOXA1, SALL2, CUL3, DURS1, CA8, FOXL2, COL25A1, CPA6, FGF9, PHOX2A, CECR2, CDX2, IFNG, ARX, CREBBP, KIR2DL1, HLA-DRB5, SCD5, MOCS3, BCAS4, KIR2DL4, KIR3DL3, CHRNB3, REV3L, PLXND1, PIEZO2, MBS1, MBS3, MT-TA, EYA1, FGF13, EP300,	ROBO3, PHOX2A, KIF21A, UBC CHL1, ELOC, ELOB, CHL1-AS2, ROBO3	TIMP2, TGFBI

L1CAM, DRD2, GJA1, NEUROG1, EPHA4, STAT1, KIR3DP1, HOXD3, GZMB, UBA2, ECEL1, ADAM17, PTPRN2, ROBO3, TOLLIP, XIRP2, BTRC, LBX1, HGS, HOXD1		

Table 2: Identification of Hub proteins

Degree Centrality		**Betweenness Centrality**		**Closeness Centrality**	
Gene	Score	Gene	Score	Gene	Score
DRD2	3560.0	DRD2	1.3852736E8	EGFR	1.2288813E-4
DRD2	3226.0	DRD2	9.7794936E7	ARRB2	1.2288793E-4
UBC	1609.0	UBC	6.9540432E7	UBC	1.2288745E-4
ADAM17	1273.0	ADAM17	4.7671964E7	DRD2	1.2288734E-4
PHAZE	1215.0	CUL3	4.2251952E7.0	CALM2	1.2288698E-4
UBC	1188.0	EGFR	3.4354112E7	CALM2	1.2288698E-4
CUL3	1176.0	UBC	2.9233554E7	CALM3	1.2288698E-4
Ubc	817.0	UBC	2.7864122E7	CUL3	1.2288621E-4
AI194771	785.0	UBC	2.567804E7	FLINA	1.2288503E-4
UBC	719.0	ARRB2	2.0969964E7	HGS	1.2288491E-4

Table 3: Module analysis

Module 1	**Module 2**	**Module 3**	**Module 4**	**Module 5**	**Module 6**	**Module 7**
ASB-3, ASB7, C2orf5, CCT-epsilon, CCT2, CCT3, CCTETA, COPS6, CUL4A, Cctq, EWS, GFPT2, HSAL2, KIF21A, MRXHF2, NCOA-62, OABP, OCT4, P1.1-MCM3, PHA2E, POP1, RP11-435J19.1, SIII, SRB, TCP20, p59ILK	ARRB2, GRB2, HGS, NEDD8, TRAF1, UBC	DmelCG12081, CG9291, CG4204, l(3)neo31	Bor, BB138287, Neurog1	CUL3, DAPK1, Kelch-like ECT2-interacting protein, PIG53, Protein pp110, TSG101, UBC	CBP, Early E1A 32 kDa protein, KAT2B	SIII p15, SIII p18, Ywhae

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Table 4 : Enrichment results

Pathway or Process	XD-score	Q-value	Overlap/size
Dorso-ventral axis formation	0.18515	1.00000	1/20
Phototransduction	0.16697	1.00000	1/22
Olfactory transduction	0.14515	1.00000	1/25
Bladder cancer	0.09042	1.00000	1/38
Nucleotide excision repair	0.08039	1.00000	1/42
Notch signaling pathway	0.08039	1.00000	1/42
Endometrial cancer	0.06515	1.00000	1/50
Non-small cell lung cancer	0.06358	1.00000	1/51
Acute myeloid leukemia	0.06208	1.00000	1/52
Endocytosis	0.05257	1.00000	3/178

Table 5: Key transcription factors

#	**Transcription Factor**	**Description**	**Target Genes**	**Mode of Regulation**
1	CIITA	class II, major histocompatibility complex, transactivator	CREBBP	Unknown
			HLA-DRB5	Unknown
			STAT1	Unknown
2	TFAP4	transcription factor AP-4 (activating enhancer binding protein 4)	IFNG	Unknown
			SALL2	Activation
3	RFX5	regulatory factor X, 5 (influences HLA class II expression)	HLA-DRB5	Unknown
			IFNG	Unknown
4	SOX2	SRY (sex determining region Y)-box 2	BMP4	Repression
			TUBB3	Repression
5	POU5F1	POU class 5 homeobox 1	BMP4	Repression
			CDX2	Repression
6	REST	RE1-silencing transcription factor	STAT1	Repression
			TUBB3	Repression
			TUBB3	Unknown
7	GATA3	GATA binding protein 3	CDX2	Activation
			IFNG	Unknown
8	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	IFNG	Repression
			SALL4	Activation
			STAT1	Activation

9	JUN	jun proto-oncogene	GJA1	Activation
			IFNG	Activation
			IFNG	Unknown
			TIMP2	Unknown
10	EP300	E1A binding protein p300	CREBBP	Unknown
			IFNG	Activation

In this study, a total of 76 DRS, HGPPS and CFEOM3 associated genes were identified from GeneCards, STICH and CTD databases (Table 1) and their protein-protein interaction network was constructed (Figure 1). Based on Betweenness Centrality, Closeness Centrality, and Degree Centrality scores DRD2, UBC and CUL3 were established as hub nodes in the Protein-Protein Interaction network of these genes and their interactions (Table 2). Seven distinct modules (Module 1, Module 2, Module 3, Module 4, Module 5, Module 6 and Module7) of the PPI network were identified (Figure 2). Dorso-ventral axis formation, Phototransduction, Olfactory transduction, Bladder cancer and Nucleotide excision repair were the top 5 pathways/processes the proteins in these modules were found to be significantly involved in (Table 3). Top 10 transcription factors targeting DRS, HGPPS and CFEOM3 associated genes have been identified from TRRUST database of transcription factors (Table 5).

One of the key players identified in this study is the D2 dopamine receptor (DRD2). It is one of the most extensively studied genes implicated in neuropsychiatric disorders. Since its association with the TaqI A DRD2 minor (A1) allele with severe alcoholism in 1990, a number of studies have been carried out (Noble 2003). DR2 have been found to be associated with schizophrenia (Shaikh et al. 1994), posttraumatic stress disorder (PTSD) (Comings et al. 1991, 1996), movement disorders (Planté-Bordeneuve et al. 1997) and so on. UBC is responsible for maintaining cellular ubiquitin levels under stress conditions (Ryu et al. 2007). Transcription of UBC gene is induced during times of stress and helps removing damaged/unfolded proteins (Ryu et al. 2007). Disruption of ubiquitination a mechanism which controls many aspects of neuronal function by regulating protein abundance in neurons has been demonstrated in neurological disorders such as Parkinson's disease, Amyotrophic Lateral Sclerosis and Angleman Syndrome (Hallengren, Chen, and Wilson 2013). CUL3 has been found to be a key regulators of sleep homeostasis and a dopamine arousal pathway in drosophila (Pfeiffenberger and Allada 2012). CUL3 is a member of the Cullin family of proteins whose job is to function as scaffold proteins of E3 ubiquitin ligase complexes. One study showed revealed cell-autonomous involvement of CUL3 in axonal arborization and dendritic elaboration of Drosophila mushroom body neurons. CUL3 mutant neurons were found to be defective in terminal morphogenesis of neurites (Zhu et al. 2005).

Therefore, the three key proteins identified in this study had been implicated in various neurological functions and disorders before but their role in axon guidance related disorders were not elucidated. This new result from the perspective of Systems Biology will hopefully inspire *in vitro* investigators to extract further information about the role of these proteins in propagating diseases like DRS, HGPPS and CFEOM3.

Conclusion

To conclude, a total of 76 DRS, HGPPS and CFEOM3 associated genes have been identified. DRD2, UBC and CUL3 have been revealed as key players in DRS, HGPPS and CFEOM3 associated pathways.

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