GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Investigation of genes in chronic and acute morphine-treated mice using microarray datasets

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Genet. Mol. Res. 14 (3): 10193-10205 (2015)
Received January 7, 2015
Accepted June 22, 2015
Published August 28, 2015
DOI http://dx.doi.org/10.4238/2015.August.28.3

ABSTRACT. Morphine is a psychoactive medication that is used as a standard analgesic treatment to relieve pain in clinics. Many patients rely on chronic or acute treatment of morphine to treat pain. However, morphine is a narcotic and has a reverse effect when inappropriately used. Therefore, it is necessary to study chronic and acute morphine treatment to improve pain relief. In this study, differentially expressed genes of acute and chronic morphine-treated mice were identified using Array Express datasets. The genes that were associated with these two types of morphine treatment are discussed. A co-expression network was constructed, and the hub genes were identified. Gene ontology enrichment analysis and pathway analysis were performed using the Gene Ontology website and Kyoto Encyclopedia of Genes and Genomes, respectively. Our study revealed genes that are associated with acute and chronic morphine treatment. Therefore, this study is

potentially useful for improving pain relief of patients by acute and chronic morphine treatment.

Key words: Chronic morphine treatment; Acute morphine treatment; Gene

INTRODUCTION

Morphine is an important psychoactive chemical in opium and is used to relieve intense pain and suffering in clinical patients. It has been considered a benchmark of analgesics for its direct action on the central nervous system. Thus, many patients experiencing pain depend on chronic morphine treatment for pain relief. However, when inappropriately used, morphine has a reverse effect and causes rapidly worsening pain (Fenu et al., 2014). In recent years, many researchers have studied the influence of morphine on human biological processes. Quanhong et al. (2012) provided a novel adjuvant for morphine treatment (acute and chronic) via investigating PLCb3 factor administration in the morphine tolerance signal pathway Belkaï et al. (2013) used acute morphine treatment to study the effect of acute methadone and buprenorphine treatment on pain management and opioid addiction at the gene level. Chen et al. (2014) found that, after chronic morphine treatment, the expression of spinal G protein was upregulated. Additionally, this effect was attenuated by knockdown of the spinal mGlu5 receptor with antisense oligonucleotides. Joshi et al. (2014) evaluated the influence of morphine treatment on elevated plus maze test parameters, oxidative stress markers, and Hsp70 expression in normal and stressed rats. Consequently, morphine was shown to differentially affect acute and chronic stress-induced changes in anxiety-related behaviors and complex interactions between oxidative stress markers and Hsp 70 expression. Umathe et al. (2012) studied the differential effect of acute and chronic morphine treatment with regard to withdrawal on obsessive-compulsive behavior. The results showed that marble burying behavior was reduced after acute morphine treatment, whereas the increase of such behaviors was shown to be closely related to the withdrawal of chronic morphine treatment (Joshi et al., 2014). Although numerous studies exist on the association of acute and chronic morphine treatment with human biological processes, research on the relationship of these two basic treatment methods is scarce. In the current study, our aim was to identify differentially expressed genes (DEGs) under acute and chronic morphine treatment. We found potential functionally defined classification genes that are related to both treatments and might provide insight into how acute and chronic morphine treatment affects biological processes. We identified differential expression profiles of acute and chronic morphine treatment using the Linear Models for Microarray Data (LIMMA) Package (Smyth, 2004) in a bioconductor. Then, the differentially co-expressed genes (DCGs) were identified, and corresponding co-expression networks were constructed. After investigating the centrality characteristics of the constructed co-expression networks, functional enrichment was performed using gene ontology (GO) (Deng and Huang, 2014) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2014). This research might provide a promising foundation for determining the effect of acute and chronic morphine treatment at the gene level.

MATERIAL AND METHODS

Preprocess of microarray datasets

A microarray dataset was extracted based on the transcription profile of E-GEOD-7762

(Korostynski et al., 2007), which was obtained from Array Express. This publicly available microarray dataset was used to evaluate DEGs between 12 subjects who were acutely treated with morphine, 12 subjects who were chronically treated with morphine, and 12 normal control subjects. The dataset was processed on a Mice430_2 platform.

For data preprocess, the probe-level data were converted into expression measures. After obtaining 45,101 genes from a dataset that was read by the Affy package, the robust multiarray average method (Ma et al., 2006) and a quartile-based algorithm (Rifai and Ridker, 2001) were used to correct background and normalize the quartile data. Additionally, the MAS5 algorithm (Pepper et al., 2007), for which the value was selected via the median method, was applied to determine perfect match and mismatch values (Lacher et al., 2014). Gene screening was then carried out based on the nsFilter method of the Gene Filter package. Genes with inter-quartile ranges (Martin, 2004) greater than 0.5 were discarded. The platform annotation files that were provided by Affymetrix Company were used to map the relationship between the probes and gene symbols. A probe with no corresponding gene symbol would be filtered, and the most significant differentially expressed probe-set that was analyzed by the maximum-based method was retained if multiple probe-sets were associated with one gene. Finally, 10,313 probe genes were obtained.

Identification of DEGs

The DEGs were identified using the freely available R platform (http://cran.r-project.org/). The data were further screened by the topTable method (Smyth, 2004) in the LIMMA package, which is a Bayes method available through Bioconductor (http://www.Bioconductor.org/) (Gentleman et al., 2004). The maximum number of genes were set as 10,313, and P value was adjusted to *q* value using the false discovery rate method (Martin, 2014). Only the genes that met our criteria (logFC > 2 and P < 0.05) were selected as DEGs in this study. **(Links ok)**

Identification of DCGs in co-expression network

Co-expressed genes played important roles in the accomplishment of biological function and participated in similar biological pathways (Ma et al., 2014). In fact, genes with functional relationships were frequently co-expressed across the samples (Bergmann et al., 2003; Stuart et al., 2003; Lee et al., 2004).

In the current research, we used the R/EB co-express package, which uses an empirical Bayesian framework for DCG discovery (Dawson et al., 2012), to identify DCGs in mice that were treated with acute and chronic morphine compared with their corresponding normal subjects (Yang et al., 2013). The DEGs selected were applied, and the function makeMyD (Dawson, 2012) was used to convert the X expression matrix into the D correlation matrix (X refers to an m-by-n expression matrix, where rows are genes and columns are subjects; D refers to the correlation matrix output of makeMyD) by using Pearson's correlation coefficient based on a two-dimensional matrix. Then, the hub genes were calculated by rankMyGenes function (Dawson, 2012), which used a threshold to determine the names of the differently co-expressed pairs. rankMyGenes function then split those pairs into their constituent genes and created a table with the associated data. A sorted version of the table is then returned. Finally, the co-expression network was drawn by showNetwork (Dawson, 2012), a function to evaluate co-expression among a small group of genes. In the constructed network, the edges were

colored based on the correlation strength, which was indicated by the D matrix that was input and ranged from red (strong negative correlation) to blue (strong positive correlation). The degree of the expressed correlation was set as 0.9 (Dawson, 2012).

Centralities of network

Stress was defined as the number of shortest paths passing through a node. High stress values indicated that the protein was involved in connecting regulatory molecules (Scardoni and Laudanna, 2012). Another parameter of centrality was betweenness, which described the core of node in the network. In present study, the thresholds of stress and betweeness were set as 1419.19 and 629.88, respectively. The centralities of the networks were analyzed by the Centiscape plug-in (Scardoni et al., 2009) via mapped DEGs into the Cytoscape software (Gonçalves, 2014).

Functional enrichment analysis

To further investigate the biological functions of these genes, GO enrichment analysis and KEGG pathway enrichment analysis were performed using an online tool, the Database for Annotation, Visualization, and Integrated Discovery (DAVID) bioinformatics resource (http://david.abcc.ncifcrf.gov/tools.jsp) (Huang et al., 2008).

DAVID bioinformatics resources could systematically extract biological meaning from a large number of genes. GO terms and KEGG pathways with a P value less than 0.05 (except for cellular components in GO terms of chronic morphine treated, $P < 0.1$) were chosen based on expression analysis systematic explorer (EASE) test (Hosack et al., 2003), which was applied in DAVID.

EASE analysis of the regulated genes indicated molecular functions and biological processes that were unique to each category (Ford et al., 2006). The EASE score was used to detect significant categories. The threshold of EASE score, <0.05, and the minimum number of genes for the corresponding term, >2, were considered to be significant for a category.

RESULTS

Identification of DEGs

After normalization and preprocessing of the expression profile dataset, 481 and 75 DEGs of acute and chronic morphine treatment, respectively, were identified using the LIMMA package based on the research criteria ($logFC > 2$ and $P < 0.05$). DEGs that were identified from the microarray datasets are shown in Table 1, and 23 common genes were selected, including *Zbtb16*, *Pkp2*, *Plin4*, *Cebpa*, *Map3k6*, *Cdkn1a*, *Camk1g*, *Tsc22d3*, *Sgk3*, *Tmem125*, *Slc2a1*, *Greb1l*, *Slc25a13*, *Gng11*, *Fkbp5*, *Wnt7a*, *Lurap1l*, *Opalin*, *Arfrp1*, *Frmd4b*, *Olig2*, *Dusp16*, and *Lap3*.

Identification of DCGs in a co-expression network

Twenty hub genes were identified and considered DCGs in mice treated with acute morphine. These genes included *Ankrd63*, *A330023F24Rik*, *Mphosph9*, *Ntrk2*, *Sf3b1*, *Tbcc*, *9330154F10Rik*, *9630021D06Rik*, *Ank3*, *Celf2*, *Clk1*, *D4Wsu53e*, *Dusp8*, *Fam21*, *Gm9983*, *Ift20*, *Lonrf3*, *Slc7a6*, *Syn2*, and *Zfp612*. A co-expression network of these 20 hub genes was drawn using the "showNetwork" function in R. However, for chronic morphine treatment DCGs, fewer hub genes were found based on empirical Bayesian algorithm. Additionally, the co-expression network was made using the top 20 DEGs that were thought to be DCGs (Figure 1).

Table 1. Identified DEGs of acute and chronic morphine treatments.

Acute morphine treatment							
No.	Symbol	No.	Symbol	No.	Symbol	No.	Symbol
1	Gm11627	121	Ppap2b	241	Fmo2	361	9630021D06Rik
2	Zfand4	122	Rufy3	242	BB211804	362	Tinagl1
3	Zbtb16	123	Lurap1l	243	Setx	363	Itga6
4	Pkp2	124	Hectd1	244	Nucks1	364	Prmt6
5	Plin4	125	Mcc	245	Usp3	365	Mier1
6	Cebpa	126	Prpf4b	246	Ifnar2	366	Gramd1c
7	Map3k6	127	Eltd1	247	Trmt11	367	Desi2
8	Cdkn1a	128	Mmab	248	Wbscr16	368	Vps72
9	Lifr	129	D4Wsu53e	249	Zc3h15	369	Lrrc57
10	Pdk4	130	Ezh2	250	Npr3	370	Celf2
11	Aff1	131	2700050L05Rik	251	Vcan	371	Ywhab
12	Camk1g	132	Cdadc1	252	Gspt2	372	Pcolce2
13	Tsc22d3	133	Pnpla7	253	BC018507	373	Psme4
14	Coa6	134	Add2	254	Brd4	374	Rbl2
15	Adipor2	135	Sf3b1	255	Sesn1	375	Ntrk2
16	Sgk3	136	Camk1d	256	Flcn	376	Arap2
17	Tfcp2l1	137	Adcy9	257	Arhgef2	377	Clmn
18	Heph	138	AI503316	258	Plekho2	378	Fam135a
19	Tanc1	139	Opalin	259	Osbpl11	379	Eif4g1
20	Cx3cr1	140	Phka1	260	Hlf	380	Smpd3
21	Lhfp	141	Wasl	261	Paqr5	381	Mttp
22	1810011O10Rik	142	Gpr83	262	Pycard	382	Orai2
23	Snx1	143	Ankrd63	263	Cntnap2	383	Zfp612
24	Azin1	144	Arhgap18	264	Ndufab1	384	B930095G15Rik
25	A330023F24Rik	145	Atp11a	265	Gabrr2	385	Herc6
26	Prss23	146	Smim3	266	Slc41a1	386	Clcn4-2
27	Mfsd2a	147	Vav2	267	Ahnak	387	Rnft1
28	Dock9	148	Arl13b	268	Syn2	388	Irak1
29	Tmem125	149	Atp5c1	269	Ryr3	389	Pcsk7
30	St8sia3	150	Emilin2	270	Hras1	390	Ubp1
31	Usp54	151	Dnajc28	271	Usp53	391	Notch4
32	Slc2a1	152	Fam169a	272	Rad1	392	Atp7a
33	Mpp7	153	AI956758	273	Hspa8	393	Sgpp2
34	Ppp1r2	154	Ikzf2	274	Lrrc20	394	Akap9
35	Lonrf3	155	Dlg5	275	Rab6b	395	Ankrd39
36	Lrrc8c	156	Pltp	276	Traf7	396	Plekhg4
37	5031426D15Rik	157	Ino80c	277	Fubp1	397	Mthfr
38	Fzd2	158	Ttc21b	278	Frmd4b	398	1810041L15Rik
39	Cmtm3	159	Cd164	279	Stxbp3a	399	Rell1
40	2900046F13Rik	160	Ank3	280	Dhdh	400	9030425L15Rik
41	Rhpn2	161	Cacna1d	281	Lrrc8a	401	Sgtb
42	Pdlim1	162	Tmem5	282	Fnbp4	402	Dbp
43	Nt5e	163	Idua	283	Lrtm2	403	5530601H04Rik
44	B3galnt2	164	Lysmd1	284	Kcnd2	404	Sfxn2
45	Greb1l	165	Khdrbs2	285	Trank1	405	Ppm1e
46	C030009J22Rik	166	Col4a2	286	Dlgap2	406	Foxo1
47	Igfbp3	167	Tst	287	Dio2	407	Bsg
48	Mlxip	168	Rbm12b1	288	Wnk1	408	Irs1
49	Taf2	169	Ptprg	289	Galnt15	409	Zfand5
50	Ptpre	170	Gnai1	290	Bbip1	410	Pde10a
51	Tekt4	171	Plekhf1	291	Synj2bp	411	Tcp11l2
52	Nostrin	172	Tbcc	292	BB283564	412	Mllt10
53	Abcc4	173	H2afx	293	Olig2	413	Tmod3
54	Itpk1	174	Cklf	294	Smarcd2	414	Pars2
55	Dram1	175	Ift20	295	Prkx	415	Kifap3
56	Atp10a	176	Vldlr	296	Asb11	416	Fgf3
57	Slc25a13	177	Fam21	297	G2e3	417	Mdn1
58	Aass	178	Arfrp1	298	Zbtb7a	418	5730458M16Rik
59	Gng11	179	Cspp1	299	Slc7a6	419	Ece1
60	Rala	180	A930011O12Rik	300	Flywch1	420	Tor1aip2

Continued on next page

Table 1. Continued.

Acute morphine treatment							
No.	Symbol	No.	Symbol	No.	Symbol	No.	Symbol
61	Grm1	181	Necab1	301	Adamts9	421	Snd1
62	Clk1	182	Kcna5	302	B830032F12	422	Zfand2a
63	Frmpd1	183	Ago3	303	Ssh2	423	2310039F13Rik
64	Tma16	184	Clip1	304	Dis3l2	424	Pura
65	Fzd7	185	Sema5a	305	Cacng4	425	C030023E24Rik
66	Slc8a3	186	Rbmx	306	Dnajc1	426	Ssr4
67	Mxd4	187	Rab31	307	Mphosph9	427	Cx3cl1
68	Gjb6	188	Arhgef17	308	Cobl	428	Baz2a
69	Osgin2	189	Crot	309	Gm9983	429	Amy1
70	Bcl6	190	Pdpk1	310	Dusp16	430	Fam171a2
71	Fnbp1	191	Tnfrsf25	311	Sbno1	431	Dact1
72	Cxxc5	192	Mdm4	312	Filip1l	432	Skp2
73	Ranbp9	193	Bcr	313	Arhgef28	433	Ppip5k2
74	Ftx	194	Nfe2l2	314	Nipal4	434	Npas4
75	Tmem98	195	Gpr22	315	Fyn	435	Pcp4l1
76	Ccdc6	196	Atxn2	316	Letmd1	436	Epha10
77	Htra1	197	Slc6a20a	317	5430440L12Rik	437	Gli3
78	Eva1b	198	Ndufa10	318	Vstm5	438	Scn1a
79	Fn1	199	Cdc37l1	319	Smarcc1	439	G3bp2
80	Ccdc141	200	Cpeb3	320	Lhpp	440	Cacna1g
81	Trp53inp1	201	Adam8	321	Herpud1	441	Tnrc18
82	Sgk1	202	Zfp719	322	Cntnap4	442	Nacc2
83	Gpatch8	203	Nr3c2	323	Vip	443	Fam136a
84	Pla2g3	204	Dab2ip	324	Tapt1	444	Prima1
85	Sult1a1	205	Nav3	325	Ankrd16	445	Casp2
86	Mfsd11	206	Chd4	326	Ogfr	446	Gjc2
87	Aldh6a1	207	Acot11	327	D10Bwg1379e	447	Nr2c2ap
88	Inpp5f	208	Ubxn7	328	Nfkbiz	448	Nxn
89	Map6d1	209	Dgkg	329	Pld1	449	Cpne7
90	Pak7	210	Dusp8	330	Ubtf	450	Ampd3
91	Ppp2r3a	211	Fosl2	331	Chfr	451	Ahctf1
92	Zfp410	212	Ccdc85a	332	Cxadr	452	Nmt2
93	Fkbp5	213	Zfp608	333	Sap30	453	6430604M11Rik
94	Dpf1	214	Wipf3	334	Ucp2	454	Wwc1
95	Tsc22d1	215	Kndc1	335	H2afy	455	Itga8
96	Spns2	216	Klf4	336	Per2	456	1110057K04Rik
97	Pou3f1	217	Ccnl2	337	Txnrd1	457	Snx24
98	Csnk2a1	218	Lrrc58	338	Tnfrsf19	458	Spred2
99	Pxdn	219	Zwilch	339	Gal3st1	459	Dnajc3
100	Slc16a6	220	Bicd2	340	Suox	460	Atp6v0a2
101	Alkbh1	221	Arhgef3	341	P4ha2	461	Fam20a
102	Ctla2a	222	Nfat5	342	Pkp4	462	Magi2
103	Ldb2	223	Acsl3	343	Mat2a	463	Akap12
104	Tm6sf1	224	Pten	344	Fam20c	464	Cdk7
105	Cyp4v3	225	Tmem88b	345	Rps6kb2	465	Snw1
106	Wnt7a	226	Nek1	346	Cacnb1	466	Orc2
107	Arhgap21	227	Ifrd1	347	Gm15417	467	Dusp6
108	Ogt	228	Fgfrl1	348	Prodh	468	Fbxl12
109	Kdsr	229	D1Ertd448e	349	Fdft1	469	Ctgf
110	Gprin3	230	Tm6sf2	350	LOC101056642	470	Klf15
111	Zfp91	231	Kank3	351	Slc9a2	471	Fzd1
112	9330154F10Rik	232	Acsbg1	352	Lap3	472	Tesc
113	Plxdc2	233	Rasal1	353	P2ry13	473	Lcmt2
114	Gkn3	234	Grm3	354	Ppp4r1	474	Snord89
115	Pnpla2	235	Prpf39	355	2610011E03Rik	475	Gabra4
116	Mak	236	Cp	356	D5Wsu148e	476	Gm2590
117	D930009K15Rik	237	Sphkap	357	Slc1a3	477	Secisbp2l
118	Tnpo1	238	9530057J20Rik	358	2700062C07Rik	478	9230110C19Rik
119	Rin2	239	Ier5l	359	Fam134b	479	Bbs5
120	Plat	240	Mtm1	360	Tgm2	480	2210404J11Rik

Continued on next page

Table 1. Continued.

Chronic morphine treatment							
No.	Symbol	No.	Symbol	No.	Symbol	No.	Symbol
1	Zbtb16	20	Arhgap36	39	Neurod2	58	C030034E14Rik
2	Plin4	21	Slc16a1	40	Vat1l	59	Rhou
3	Camk1g	22	Acot1	41	Wscd1	60	Fkbp5
4	Slc25a13	23	Cd24a	42	Sh3rf1	61	Ccdc106
5	Gng11	24	Greb1l	43	Tsga10	62	Tcirg1
6	Cdkn1a	25	Lurap1l	44	Lypd1	63	Opalin
7	Tsc22d3	26	Sdk2	45	Nkx2-2	64	Tuba8
8	Pkp2	27	Aspa	46	Map3k6	65	Emp2
9	Rgs10	28	Kcne1l	47	Clspn	66	Tspan6
10	Ido1	29	Ugt8a	48	Frmd4b	67	Nr4a2
11	Map2k3	30	Cebpa	49	Dusp16	68	C030011L09Rik
12	Enox2	31	Olig2	50	Tmem125	69	Cbx4
13	Dnah1	32	Dusp4	51	Gpr155	70	Sncaip
14	Sgk3	33	Fabp7	52	St8sia2	71	Dock11
15	Wnt7a	34	Lap3	53	Arfrp1	72	AI314604
16	Rfx4	35	Slc35d3	54	Nexn	73	Lgmn
17	Bhlhe40	36	Mtss1	55	Ctxn2	74	1700007K13Rik
18	Evi2a	37	BC005764	56	Tef	75	Slc46a1
19	Insl6	38	Slc2a1	57	Ldlrad3		

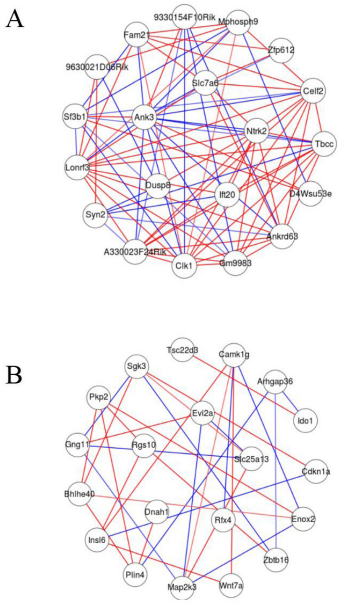

Figure1. Co-expression network of acute morphine treatment (**A**) and chronic morphine treatment (**B**). Nodes are genes. Edges are gene pairs, with color indicating strength of correlation, ranging from blue (negative correlation) to white (uncorrelated) and to red (positively correlated).

Functional enrichment analyses

GO analyses were carried out in three categories, including biological processes (BP), molecular functions (MF), and cellular components (CC). For GO enrichment analysis of acute morphine-treated mice, the 481 DEGs were significantly enriched in 54 BP terms, 25

MF terms, and 18 CC terms. Alternatively, for GO enrichment of the chronic morphine treated subject, the 75 DEGs were significantly enriched in 35 GO BP terms, 7 MF terms, and 4 CC terms. After discarding the terms with P values less than 0.01, the five most significant GO BP, MF, and CC terms are presented in Table 2. The most significant BP, MF, and CC terms pertaining to acute morphine-treated subjects were phosphate metabolic process ($P < 0.01$), Rasguanyl-nucleotide exchange factor activity ($P < 0.01$), and plasma membrane ($P < 0.01$), respectively. For the chronic morphine-treated subjects, the most significant corresponding BP, MF, and CC terms were negative regulation of cell proliferation ($P < 0.01$), transcription regulator activity ($P < 0.05$), and adherents junctions ($P = 0.06$), respectively. The top four significant terms of GO analysis are shown in Table 2.

For KEGG pathway enrichment analysis of acute morphine-treated mice, our results showed that the DEGs were significantly enriched in eight pathways (Table 3). Alternatively, for the chronic morphine-treated subjects, the DEGs were significantly enriched in the KEGG pathway terms in cancer. These DEGs are involved in both of the acute and chronic KEGG terms.

DISCUSSION

Morphine, which is a mainstay in pain management, has been used for pain treatment for more than 5000 years (Quanhong et al., 2012). In the present study, we identified DEGs in chronic and acute morphine-treated subjects by secondary use of multiple microarray data. We then analyzed the DEGs using a network-based algorithm. The correlation between mRNA expression in chronic and acute morphine-treated subjects was characterized, and the results indicated that the common genes that correspond to DEGs might be significant.

To date, several common genes have been reported with regard to morphine treatment. *Tsc22d3*, which is a gene that encodes TSC22 domain family protein 3 (Vogel et al., 1996), was identified as a co-expressed gene of transcriptional activation in inbred mouse strains that were administrated acute and chronic morphine by Korostynski et al. (2007). There were differences between strains with regard to the magnitude of transcriptional response to acute morphine treatment, and the degree of gene expression tolerance relative to chronic morphine treatment was observed (Korostynski et al., 2007). *Tsc22d3* has also shown to be a morphine-responsive gene for knockdown that results in alterations to dendritic spines that possibly reflects an altered potential for plastic changes in the mouse striatum (Piechota et al., 2010). Additionally, *Fkbp5*, which is a drug-responsive gene that codes for FKBP5, has a role in posttraumatic stress disorder, depression, and anxiety by genetic studies (Binder, 2009). Szklarczyk et al. (2012) found that traumatic stress induced the upregulation of *Tsc22d3* and expression of *Fkbp5*. Moreover, traumatic stress further enhanced sensitivity to the rewarding properties of morphine.

The results of this research demonstrated that *Tsc22d3* and Fkbp5 related closely with morphine treatment (Szklarczyk et al., 2012). *Olig2* was identified as a novel morphine-regulated gene (Korostynski et al., 2007), because the expression of *Olig2* was enhanced when the subject was exposed to morphine (Hahn et al., 2012). The hub genes of acute chronic treated subjects were also considered to have potential significance. *Ntrk2*, a neural differentiation- and growth-associated gene, experienced dynamic changes upon morphine exposure (Wen et al., 2013). The genes mentioned above that are reported to change when exposed to morphine treatment are probably genes that are related to both chronic and acute morphine treatment. Therefore, these genes may be useful for developing novel approaches for researchers to study the impacts on biological functions in people who are treated with morphine.

Table 2. Significant terms of GO enrichment.

Terms	P value	Counts
Acute morphine treatment		
BP		
Phosphate metabolic process	0.0005	PPP2R3A, PRPF4B, NEK1, SSH2, RPS6KB2, EPHA10, CLK1, PTEN, PRKX, MTM1, PAK7, MAP3K6, PDPK1, CSNK2A1, DUSP16, BRD4, IRAK1, SGK1, CAMK1G, PTPRE, BCR, SGK3, PTPRG, MAK, PDK4, WNK1, CDK7, GRM1, PPM1E, FYN, NTRK2, ATP5C1, IGFBP3, DUSP8, ATP6V0A2, DUSP6, CAMK1D
Phosphorus metabolic process	0.0005	PPP2R3A, PRPF4B, NEK1, SSH2, RPS6KB2, EPHA10, CLK1, PTEN, PRKX, MTM1, PAK7, MAP3K6, PDPK1, CSNK2A1, DUSP16, BRD4, IRAK1, SGK1, CAMK1G, PTPRE, BCR, SGK3, PTPRG, MAK, PDK4, WNK1, CDK7, GRM1, PPM1E, FYN, NTRK2, ATP5C1, IGFBP3, DUSP8, ATP6V0A2, DUSP6, CAMK1D
Regulation of cell proliferation	0.0008	FOSL2, PPP2R3A, FGFRL1, FOXO1, ZBTB16, CXADR, PTEN, GLI3, ZFP91, KIFAP3, TGM2, BCL6, FGF3, CEBPA, TESC, LIFR, GJB6, IRS1, PURA, HRAS1, CDKN1A, DBP, NOTCH4, MDM4, IGFBP3, KLF4
Regulation of programmed cell death	0.0013	FOXO1, ZBTB16, CX3CL1, PTEN, GLI3, PAK7, ZFP91, G2E3, TSC22D3, PYCARD, TGM2, TRP53INP1, BCL6, DLG5, TRAF7, CASP2, SGK3, SKP2, HRAS1, PLEKHF1, ATP7A, CDKN1A, CX3CR1, ALKBH1, IGFBP3, CAMK1D
CC		
Plasma membrane	0.0059	SLC8A3, GPR83, SLC6A20A, FGFRL1, CXADR, SLC7A6, ARHGAP21, SLC1A3, SLC2A1, SPRED2, RALA, ELTD1, DLG5, ADAM8, NT5E, AHNAK, BSG, PLD1, DAB2IP, ARHGEF2, MAGI2, CAMK1G, KCND2, LIFR, TANC1, MPP7, PNPLA2, SYNJ2BP, CD164, GRM1, HRAS1, GABRR2, GRM3, PLXDC2, CX3CR1, ADD2, BBS5, SCN1A, TNFRSF25, SSH2, PHKA1, CACNB1, SNX1, NOSTRIN, GNG11, EPHA10, KCNA5, ZBTB16, CX3CL1, GJC2, GPR22, ECE1, PLIN4, OPALIN, SYN2, TGM2, TNFRSF19, TRAF7, PRIMA1, FN1, GABRA4, DLGAP2, FRMPD1, GJB6, VAV2, ATP7A, P2RY13, FNBP1, RAB31, ADCY9, ITGA6, FYN, PKP2, ITGA8, NOTCH4, PKP4, NTRK2, CACNA1G, ABCC4, STXBP3A, CP, CACNA1D, ATP6V0A2
Cell junction	0.0076	SCN1A, ARHGEF2, MAGI2, GABRA4, DLGAP2, SSH2, TANC1, MPP7, GJB6, CXADR, GJC2, GABRR2, ARHGAP21, ITGA6, PKP2, PKP4, SYN2, DLG5, PRIMA1, AHNAK
Cytoplasmic vesicle	0.0084	PLAT, BSG, SGK3, YWHAB, NOSTRIN, ACSBG1, ARHGAP21, ATP7A, FNBP1, PDPK1, ECE1, SND1, SLC2A1, SYN2, SPRED2, ABCC4, RAB6B, TRAF7, HSPA8, ATP6V0A2, MAP6D1
Cytoplasmic membrane-bounded vesicle	0.0098	PLAT, BSG, SGK3, YWHAB, NOSTRIN, ACSBG1, ATP7A, FNBP1, PDPK1, ECE1, SND1, SLC2A1, SYN2, ABCC4, TRAF7, HSPA8, ATP6V0A2, MAP6D1
MF		
Ras guanyl-nucleotide exchange factor activity	0.0001	PLEKHG4, ARHGEF3, ARHGEF2, BCR, WBSCR16, KIFAP3, DOCK9, ARHGEF17, VAV2, KNDC1
Rho guanyl-nucleotide exchange factor activity	0.0010	PLEKHG4, ARHGEF3, ARHGEF2, BCR, KIFAP3, DOCK9, ARHGEF17, VAV2
Guanyl-nucleotide exchange factor activity	0.0016	PLEKHG4, ARHGEF3, ARHGEF2, BCR, WBSCR16, KIFAP3, DOCK9, ARHGEF17, D10BWG1379E, VAV2, KNDC1
GTPase regulator activity	0.0029	ARHGEF3, ARHGEF2, DAB2IP, BCR, WBSCR16, DOCK9, ARHGEF17, ARHGAP18, VAV2, ARHGAP21, PLEKHG4, RASAL1, KIFAP3, RIN2, WASL, D10BWG1379E, ARAP2, KNDC1
Chronic morphine treatment		
BP		
Negative regulation of cell proliferation	0.0092	CEBPA, CDKN1A, IDO1, ZBTB16, CD24A
Spinal cord oligodendrocyte cell differentiation	0.0110	OLIG2, NKX2-2
Spinal cord oligodendrocyte cell fate specification	0.0110	OLIG2, NKX2-2
Negative regulation of apoptosis	0.0114	CDKN1A, TSC22D3, SGK3, NR4A2, IDO1
CC		
Adherens junction	0.0601	PKP2, NEXN, RHOU
Anchoring junction	0.0779	PKP2, NEXN, RHOU
Apical part of cell	0.0925	TCIRG1, LGMN, SLC46A1
Basolateral plasma membrane	0.0983	SLC2A1, NEXN, RHOU
MF		
Transcription regulator activity	0.0181	CEBPA, TSC22D3, RFX4, NEUROD2, NR4A2, TEF, BHLHE40, OLIG2, ZBTB16, NKX2-2
Structure-specific DNA binding	0.0273	CLSPN, TEF, ZBTB16
Protein dimerization activity	0.0275	CEBPA, NR4A2, TEF, OLIG2, ZBTB16
MAP kinase phosphatase activity	0.0399	DUSP4, DUSP16

Table 3. Significant KEGG pathway.

Terms	P value	Counts
Acute morphine treatment		
Pathways in cancer	0.0002	CEBPA, COL4A2, BCR, SKP2, FZD1, FOXO1, ZBTB16, FZD2, PTEN, GLI3, FZD7, HRAS1, CCDC6, CDKN1A, ITGA6, SLC2A1, RALA, WNT7A, FGF3, FN1
Melanogenesis	0.0085	ADCY9, GNAI1, FZD1, FZD2, WNT7A, PRKX, FZD7, HRAS1
MAPK signaling pathway	0.0086	CACNB1, CACNG4, PRKX, HRAS1, MAP3K6, DUSP16, NTRK2, CACNA1G, PLA2G3, DUSP8, CACNA1D, HSPA8, FGF3, DUSP6
Sphingolipid metabolism	0.0160	SGPP2, KDSR, PPAP2B, SMPD3, GAL3ST1
Dilated cardiomyopathy	0.0201	ITGA6, ADCY9, ITGA8, CACNB1, CACNG4, CACNA1D, PRKX
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.0302	ITGA6, PKP2, ITGA8, CACNB1, CACNG4, CACNA1D
Basal cell carcinoma	0.0388	FZD1, FZD2, GLI3, WNT7A, FZD7
Focal adhesion	0.0413	PAK7, PDPK1, COL4A2, ITGA6, FYN, ITGA8, VAV2, PTEN, FN1, HRAS1
Chronic morphine treatment		
Pathways in cancer	0.0373	CEBPA, CDKN1A, SLC2A1, ZBTB16, WNT7A

GO addresses the obstacle of genomic database interoperability and produces a novel, dynamic, and controlled vocabulary that can be applied to all eukaryotes. Three unique ontologies constructed on the internet (http://www.geneontology.org) are BP, MF, and CC. Among them, BP refers to genes or gene products that alter biological processes by involving a chemical or physical transformation and is accomplished by one or more ordered MF assemblies (Ashburner et al., 2000). A total of 16 BP terms (Table 4) are involved in both of acute and chronic subjects, including phosphate metabolic process, phosphorus metabolic process, regulation of cell proliferation, and regulation of programmed cell death. The most significant term for the chronic subjects was negative regulation of cell proliferation, which had a P value < 0.01, was also significant for the acute subjects, which had a P value < 0.01. Regarding MF, three terms including protein dimerization activity ($P < 0.01$), MAP kinase phosphatase activity ($P < 0.05$), and MAP kinase tyrosine/serine/threonine phosphatase activity ($P < 0.05$) were significant for both the acute and chronic morphine-treated subjects.

Table 4. Common GO terms of acute and chronic morphine treatment.

Terms	P value	Counts
BP		
Regulation of programmed cell death	0.0013	FOXO1, ZBTB16, CX3CL1, PTEN, GLI3, PAK7, ZFP91, G2E3, TSC22D3, PYCARD, TGM2, TRP53INP1, BCL6, DLG5, TRAF7, CASP2, SGK3, SKP2, HRAS1, PLEKHF1, ATP7A, CDKN1A, CX3CR1, ALKBH1, IGFBP3, CAMK1D
Regulation of cell death	0.0015	FOXO1, ZBTB16, CX3CL1, PTEN, GLI3, PAK7, ZFP91, G2E3, TSC22D3, PYCARD, TGM2, TRP53INP1, BCL6, DLG5, TRAF7, CASP2, SGK3, SKP2, HRAS1, PLEKHF1, ATP7A, CDKN1A, CX3CR1, ALKBH1, IGFBP3, CAMK1D
Negative regulation of cell proliferation	0.0022	CEBPA, TESC, PPP2R3A, FGFRL1, GJB6, ZBTB16, CXADR, GLI3, PTEN, CDKN1A, KIFAP3, BCL6, IGFBP3, KLF4
Regulation of apoptosis	0.0024	FOXO1, ZBTB16, CX3CL1, PTEN, GLI3, PAK7, G2E3, TSC22D3, PYCARD, TGM2, TRP53INP1, BCL6, TRAF7, DLG5, CASP2, SGK3, SKP2, HRAS1, PLEKHF1, ATP7A, CDKN1A, CX3CR1, ALKBH1, IGFBP3, CAMK1D
Negative regulation of apoptosis	0.0099	SGK3, SKP2, FOXO1, CX3CL1, PTEN, HRAS1, PAK7, TSC22D3, CDKN1A, G2E3, CX3CR1, BCL6, CASP2
Negative regulation of programmed cell death	0.0116	SGK3, SKP2, FOXO1, CX3CL1, PTEN, HRAS1, PAK7, TSC22D3, CDKN1A, G2E3, CX3CR1, BCL6, CASP2
Negative regulation of cell death	0.0119	SGK3, SKP2, FOXO1, CX3CL1, PTEN, HRAS1, PAK7, TSC22D3, CDKN1A, G2E3, CX3CR1, BCL6, CASP2
Negative regulation of transcription	0.0135	CEBPA, ZBTB7A, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, PRMT6, PER2, BCL6, MDM4, OLIG2, BAZ2A, KLF4
Regulation of kinase activity	0.0141	IRAK1, CDKN1A, DGKG, DUSP16, SPRED2, TRAF7, DNAJC3, VAV2, GRM1, IRS1, VLDLR

Continued on next page

Table 4. Continued.

Terms	P value	Counts
Regulation of transferase activity	0.0176	IRAK1, CDKN1A, DGKG, DUSP16, SPRED2, TRAF7, DNAJC3, VAV2, GRM1, IRS1, VLDLR
Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	0.0227	CEBPA, ZBTB7A, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, PRMT6, PER2, BCL6, MDM4, OLIG2, BAZ2A, KLF4
Negative regulation of nitrogen compound metabolic process	0.0249	CEBPA, ZBTB7A, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, PRMT6, PER2, BCL6, MDM4, OLIG2, BAZ2A, KLF4
Negative regulation of macromolecule metabolic process	0.0269	CEBPA, ZBTB7A, SKP2, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, SND1, PRMT6, PER2, BCL6, MDM4, OLIG2, IGFBP3, BAZ2A, KLF4
Negative regulation of macromolecule biosynthetic process	0.0349	CEBPA, ZBTB7A, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, PRMT6, PER2, BCL6, MDM4, OLIG2, BAZ2A, KLF4
Negative regulation of cellular biosynthetic process	0.0427	CEBPA, ZBTB7A, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, PRMT6, PER2, BCL6, MDM4, OLIG2, BAZ2A, KLF4
Negative regulation of biosynthetic process	0.0461	CEBPA, ZBTB7A, FZD1, NOSTRIN, SNW1, ZBTB16, GLI3, MXD4, PURA, SAP30, PRMT6, PER2, BCL6, MDM4, OLIG2, BAZ2A, KLF4
MF		
Protein dimerization activity	0.0091	CEBPA, HLF, FOSL2, KHDRBS2, PTPRE, IKZF2, ADIPOR2, NPR3, NPAS4, ZBTB16, SGTB, DBP, SYN2, PYCARD, NFE2L2, OLIG2
MAP kinase tyrosine/serine/threonine phosphatase activity	0.0280	DUSP16, DUSP8, DUSP6
MAP kinase phosphatase activity	0.0280	DUSP16, DUSP8, DUSP6

These results demonstrated that several mRNA expressions in chronic morphine-treated subjects were also significant for acute morphine-treated subjects. However, the result of the present study needs more support in terms of clinical evidence, and the relationship between DCGs and morphine treatment needs further evaluation. Therefore, the results of functional enrichment that we identified may merit further attention, validation, and studies.

ACKNOWLEDGMENTS

Research received no specific grants from any funding agency in public, commercial, or not-for-profit sectors

REFERENCES

Ashburner M, Ball CA, Blake JA, Botstein D, et al. (2000). Gene ontology: tool for the unification of biology. *Nat. Genet.* 25: 25-29.

Belkaï E, Crété D, Courtin C, Noble F, et al. (2013). Comparison of the transcriptional responses induced by acute morphine, methadone and buprenorphine. *Eur. J. Pharmacol.* 711: 10-18.

Bergmann S, Ihmels J and Barkai N (2003). Similarities and differences in genome-wide expression data of six organisms. *PLoS Biol*. 2: e9.

Binder EB (2009). The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology* 34: S186-S195.

Chen M, Zhang X, Xu H, Ma X, et al. (2014). Inhibitory effect of spinal mGlu(5) receptor antisense oligonucleotide on the up-regulated expression of spinal G protein associated with chronic morphine treatment. *Eur. J. Pharmacol*. 723: 253-258.

Dawson JA (2012). Package 'EBcoexpress': EBcoexpress for Differential Co-Expression Analysis. R Package version 1.12.0. Available at [http://www.biocondutor.org/packages/release/bioc/manuals/EBcoexpress/man/EBcoexpress.pdf].

Dawson JA, Ye S and Kendziorski C (2012). R/EBcoexpress: an empirical Bayesian framework for discovering differential co-expression. *Bioinformatics* 28: 1939-1940.

Deng SP and Huang DS. (2014). An integrated strategy for functional analysis of microbial communities based on gene ontology and 16S rRNA gene. *Int. J. Data Min. Bioinform.* (*in press*).

Fenu S, Espa E, Cadoni C and Di CG. (2014). Conditioned saccharin avoidance induced by infusion of amphetamine in the nucleus accumbens shell and morphine in the ventral tegmental area: behavioral and biochemical study. *Behav. Brain Res*. 269: 55-60.

Ford G, Xu Z, Gates A, Jiang J, et al. (2006). Expression Analysis Systematic Explorer (EASE) analysis reveals differential gene expression in permanent and transient focal stroke rat models. *Brain Res*. 1071: 226-236.

Gentleman RC, Carey VJ, Bates DM, Bolstad B, et al. (2004). Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*. 5: R80.

Gonçalves E (2014). Cytocopter manual: A cytoscape plug-in for training logic models, based on CellNOptR. Available at [www.cellnopt.org/cytocopter/resources/CytocopterManual.pdf].

Hahn YK, Podhaizer EM, Hauser KF and Knapp PE (2012). HIV-1 alters neural and glial progenitor cell dynamics in the central nervous system: coordinated response to opiates during maturation. *Glia* 60: 1871-1887.

Hosack DA, Dennis G Jr., Sherman BT, Lane HC, et al. (2003). Identifying biological themes within lists of genes with EASE. *Genome Biol*. 4: R70.

Huang DW, Sherman BT and Lempicki RA. (2008). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc*. 4: 44-57.

Joshi JC, Ray A and Gulati K (2014). Differential modulatory effects of morphine on acute and chronic stress induced neurobehavioral and cellular markers in rats. *Eur. J. Pharmacol*. 729: 17-21.

Kanehisa M, Goto S, Sato Y, Kawashima M, et al. (2014). Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res*. 42: D199-D205.

Korostynski M, Piechota M, Kaminska D, Solecki W, et al. (2007). Morphine effects on striatal transcriptome in mice. *Genome Biol*. 8: R128.

Lacher DW, Gangiredla J, Jackson SA, Elkins CA, et al. (2014). A novel microarray design for molecular serotyping of Shiga toxin-producing *Escherichia coli* isolated from fresh produce. *Appl. Environ. Microbiol*. 80: 4677-4682.

Lee HK, Hsu AK, Sajdak J, Qin J, et al. (2004). Coexpression analysis of human genes across many microarray data sets. *Genome Res*. 14: 1085-1094.

Ma C, Lv Q, Cao Y, Wang Q, et al. (2014). Genes relevant with osteoarthritis by comparison gene expression profiles of synovial membrane of osteoarthritis patients at different stages. *Eur. Rev. Med. Pharmacol. Sci.* 18: 431-439.

Ma L, Robinson LN and Towle HC (2006). ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. *J. Biol. Chem.* 281: 28721-28730.

Martin D (2004). Instrumentation and survey networks at the ESRF. in Eigth International Workshop on Accelerator Alignment. CERN, Geneva Switzerland.

Pepper SD, Saunders EK, Edwards LE, Wilson CL, et al. (2007). The utility of MAS5 expression summary and detection call algorithms. *BMC Bioinformatics* 8: 273.

Piechota M, Korostynski M, Solecki W, Gieryk A, et al. (2010). The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. *Genome Biol*. 11: R48.

Quanhong Z, Ying X, Moxi C, Tao X, et al. (2012). Intrathecal PLC(beta3) oligodeoxynucleotides antisense potentiates acute morphine efficacy and attenuates chronic morphine tolerance. *Brain Res*. 1472: 38-44.

Rifai N and Ridker PM (2001). Proposed cardiovascular risk assessment algorithm using high-sensitivity C-reactive protein and lipid screening. *Clin. Chem.* 47: 28-30.

Scardoni G and Laudanna C (2012). Centralities based analysis of complex networks. New Frontiers in Graph Theory. InTech Open.

Scardoni G, Petterlini M, and Laudanna C (2009). Analyzing biological network parameters with CentiScaPe. *Bioinformatics* 25: 2857-2859.

Smyth GK (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 3: 3.

Stuart JM, Segal E, Koller D and Kim SK (2003). A gene-coexpression network for global discovery of conserved genetic modules. *Science* 302: 249-255.

Szklarczyk K, Korostynski M, Golda S, Solecki W, et al. (2012). Genotype-dependent consequences of traumatic stress in four inbred mouse strains. *Genes Brain Behav*. 11: 977-985.

Umathe SN, Mundhada YR and Bhutada PS (2012). Differential effects of acute morphine, and chronic morphine-withdrawal on obsessive-compulsive behavior: inhibitory influence of CRF receptor antagonists on chronic morphine-withdrawal. *Neuropeptides* 46: 217-221.

Vogel P, Mägert HJ, Cieslak A, Adermann K, et al. (1996). hDIP - a potential transcriptional regulator related to murine TSC-22 and *Drosophila* shortsighted (*shs*) - is expressed in a large number of human tissues. *Biochim. Biophys Acta*.

1309: 200-204.
Wen A, Guo A and Chen YL (2013). Mu-opioid signaling modulates biphasic expression of TrkB and IκBα genes and neurite outgrowth in differentiating and differentiated human neuroblastoma cells. *Biochem. Biophys. Res. Commun*. 432: 638-642.
Yang J, Yu H and Liu BH (2013). Using the DCGL 2.0 package. Available at [https://cran.r-project.org/web/packages/DCGL/DCGL.pdf].