promoter assay that this sequence is critical for the response of *WFS1* to XBP1 overexpression. However, XBP1 did not directly bind to the ERSE-like sequence in a gel-shift assay. Our results demonstrate that *WFS1* has an ERSE-like sequence in its promoter and XBP1 regulates *WFS1* expression indirectly through this motif.

Materials and methods

Cell culture

We cultured SH-SY5Y cells in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St Louis, Missouri, USA) containing 10% fetal bovine serum (FBS). Mouse embryonic fibroblasts (MEFs) were generated from embryos of female heterozygous *XBP1* knockout mice (*XBP1+/–*) (Reimold *et al.* 2000) (kindly provided by Dr Laurie H. Glimcher, Harvard School of Public Health, Boston, MA, USA) mated with male *XBP1+/–* mice according to the methods described previously (Bruning *et al.* 1997), including a 3T3 protocol (Todaro and Green 1963). Embryos were dissected around embryonic day 12.5 and cultured in DMEM containing 10% FBS for more than 20 passages. All of the cells were cultured in an atmosphere of 5% CO_2 at 37°C.

Sample preparation for DNA microarray

Spliced human *XBP1* cDNA was amplified by PCR and cloned into the *Bam*HI/*Hind*III site of a pcDNA3.1 vector (Invitrogen, San Diego, CA, USA). We transfected SH-SY5Y cells cultured in a 10-cm dish with 10 μg spliced XBP1-expressing vector ($n = 5$) or control vector ($n = 5$) using Superfect (Qiagen, Valencia, CA, USA). After incubation for 48 h, cells were collected into TRIZOL reagents (Invitrogen) until use. Overexpression of spliced XBP1 was confirmed by real-time quantitative PCR.

DNA microarray

DNA microarray experiments were performed as described previously using an Affymetrix HU133A chip (Affymetrix, Santa Clara, CA, USA) (Iwamoto *et al.* 2004). In brief, we used 5 μg total RNA for RT into cDNA. Biotin-labeled cRNA was synthesized from cDNA by *in vitro* transcription. Fragmented cRNA was first applied to the Test2Chip (Affymetrix) to test the integrity of the sample, and then applied to the HU133A chip. The hybridization signal on the chip was scanned and subjected to image analysis (Affymetrix).

Microarray data analysis

The microarray raw data were processed by MAS5 (Affymetrix) and analyzed using GeneSpring software (SiliconGenetics, Redwood, CA, USA). Data were normalized by the median value. Raw data were also analyzed by the robust multiarray average (RMA) method (Irizarry *et al.* 2003), which was implemented in the module Affy of Bioconductor microarray analysis software (http://www.bioconductor.org/).

Bioinformatic analyses

The promoter regions of *WFS1* species were compared among species and the results of this comparison can be seen on the UCSC genome browser web site (http://genome.ucsc.edu/index.html). We did a search for three ERSE-like sequences – ERSE (CCAAT-N9-CCACG), a *CHOP* variant of ERSE (CCAAT-N(9)-GCACG) (Ubeda and Habener 2000), and a *WFS1* variant of ERSE (CCAAT-N(9)-CCTCG) (this study) – within the whole human genome sequence using the NCBI 35 assembly (http://www.ncbi.nlm.nih.gov/mapview/stats/BuildStats.cgi?taxid = 9606&build = 35, June 2004). Next, we determined whether or not other genes altered by XBP1 expression contain the ERSEs by examining the genomic region from the −9-kb upstream region of exon 1 to the −2-kb downstream region of the last exon. The transcription start site and the 3′-end of the transcript was based on Ensembl v34 (http://www.ensembl.org/).

Real-time quantitative PCR

To quantify mRNA levels of spliced-form *XBP1* (s*XBP1*) and *WFS1* genes in the transfected SH-SY5Y cells, we used real-time quantitative PCR with Taqman chemistry [s*XBP1* and *GAPDH* as an inner control; probes were labeled with 6-carboxy-fluorescein (FAM) and quenched by minor groove binder (MGB)] or Sybr/Green I dye (*WFS1*) according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). Single-strand cDNA was synthesized using oligo (dT) primer and SuperScript II reverse transcriptase (Invitrogen). For quantitative PCR, ABI7900HT (Applied Biosystems) was used. The PCR reaction was carried out as described previously and the relative ratios of s*XBP1* and *WFS1* to *GAPDH* were calculated (Kakiuchi *et al.* 2005). Primers (and probes) for quantitative RT–PCR were as follows: *WFS1*, 5′-ACCTGGTCG-TCCTCAATGTC and 5′-CATGAAGCACACCAGGTAGG; s*XBP1*, 5′-GGTCCAAGTTGTCCAGAATGC, 5′-GCCAGTGGCCGGGT-CT and 5′-FAM-CCTGCACCTGCTGCGGACTCAGC-MGB. A primer and probe mixture of GAPDH was obtained from Assay-on-Demand products (Applied Biosystems).

Promoter assay

We amplified each fragment of the promoter region of *WFS1* by PCR and cloned it into the *Mlu*I/*Bgl*II site of the pGL3-Basic vector (Promega, Madison, WI, USA). We transfected SH-SY5Y cells cultured in a 96-well plate (4×10^4 cells per well) with 0.6 μg DNA containing 0.25 μg reporter plasmid, 0.25 μg pcDNA3.1 vector with or without the insert of spliced *XBP1*, and 0.1 μg reference plasmid (pRL-CMV) (Promega) using SuperFect reagent (Qiagen). After a 48-h incubation, we measured luciferase activities using the Dual-Glo Luciferase assay system (Promega). The assay was performed independently three times.

Electrophoretic mobility shift assay (EMSA)

Electrophoretic mobility shift assays (EMSAs) were performed using the Light Shift Chemiluminescent EMSA Kit (Pierce, Rockford, IL, USA) according to the protocol recommended by the manufacturer. SH-SY5Y cells were transfected with vector expressing c-myc-tagged s*XBP1* and treated with 10 μM MG-132 (Sigma) for 2 h followed by nuclear extraction. For the supershift assay, nuclear extracts were prepared using low-salt lysis buffer (buffer A) and high-salt extraction buffer (buffer C) containing the protease inhibitor cocktail Complete™ (Roche, Mannheim, Germany). High-salt crude nuclear extracts were desalted into EMSA buffer as described previously (Sevinsky *et al.* 2004) except for the use of Complete™ and a Zeba™ Micro Spin Column (Pierce). The probe was biotin-labeled, double-stranded

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Table 1 Genes altered by overexpression of XBP1

	MAS			Affy					
Probe ID	Control	sXBP1	*p*-value	Control	sXBP1	*p*-value	Symbol	Map	Genbank
Up-regulated									
202908_at	1.38	2.13	4.12E-05	1.13	1.54	0.00695	**WFS1**	4p16	NM_006005
635_s_at	1.43	1.78	0.000638	1.50	1.66	0.0477	PPP2R5B	11q12-q13	L42374
203411_s_at	4.23	6.82	0.00103	4.26	5.40	0.00524	LMNA	1q21.2-q21.3	NM_005572
221728_x_at	22.44	28.93	0.00173	16.12	18.16	0.0429	–	–	AA628440
208735_s_at	2.50	3.49	0.00182	2.52	2.68	0.0417	CTDSP2	12q13-q15	AF022231
212052_s_at	3.89	5.26	0.00203	2.83	3.31	0.00149	KIAA0676	5q35.3	AB014576
60528_at	2.61	3.33	0.00252	2.08	2.48	0.0243	PLA2G4B	15q11.2-q21.3	N71116
211051_s_at	0.93	1.19	0.00261	0.80	0.92	0.0489	EXTL3	8p21	BC006363
36475_at	2.52	3.37	0.00271	2.74	3.11	0.0115	GCAT	22q13.1	Z97630
214726_x_at	4.71	5.46	0.0034	3.15	3.47	0.00386	ADD1	4p16.3	AL556041
203857_s_at	2.75	3.61	0.00373	1.68	2.18	0.00105	PDIA5	3q21.1	NM_006810
213144_at	2.90	4.13	0.00486	2.71	3.15	0.0427	GOSR2	17q21	AI074611
214951_at	0.78	1.04	0.00497	0.63	0.70	0.0487	SLC26A10	12q13	AL050358
213242_x_at	2.13	3.00	0.00548	2.42	2.64	0.0351	–	–	AB006622
214383_x_at	9.91	15.44	0.00604	7.92	9.65	0.0338	KLHDC3	6p21.1	BF063121
209325_s_at	1.34	1.95	0.00687	1.44	1.71	0.00117	RGS16	1q25-q31	U94829
218012_at	2.04	3.65	0.00704	2.14	3.18	0.00128	TSPYL2	Xp11.2	NM_022117
36084_at	3.95	5.06	0.0071	4.31	4.87	0.0435	CUL7	6p21.1	D38548
213378_s_at	3.42	4.56	0.00773	2.23	2.93	0.00221	DDX11	12p11	AI983033
203254_s_at	1.86	2.69	0.00774	1.70	1.92	0.014	TLN1	9p13	NM_006289
202418_at	6.39	8.28	0.00873	3.86	4.40	0.00709	YIF1	11q13	NM_020470
219390_at	1.87	2.64	0.00908	1.00	1.21	0.0111	FKBP14	7p15.1	NM_017946
218140_x_at	9.10	11.70	0.0114	5.78	7.32	0.000242	SRPRB	3q22.1	NM_021203
201469_s_at	5.27	6.99	0.0121	3.65	4.68	0.000432	SHC1	1q21	AI809967
214196_s_at	1.16	1.52	0.0129	0.60	0.76	0.0333	TPP1	11p15	AA602532
218058_at	4.87	6.57	0.0138	4.85	5.78	0.02	CXXC1	18q12	NM_014593
217888_s_at	2.74	3.46	0.0139	1.96	2.43	0.00386	ARFGAP1	20q13.33	NM_018209
202842_s_at	7.23	9.09	0.0143	3.45	4.34	0.0189	**DNAJB9**	7q31l14q24.2-q24.3	AL080081
209607_x_at	3.91	6.02	0.0152	2.89	3.71	0.0308	SULT1A3	16p11.2	U08032
210616_s_at	17.61	22.56	0.0179	13.34	15.48	0.00793	SEC31L1	4q21.3	AB020712
210580_x_at	4.66	6.45	0.0184	3.58	4.77	0.0248	SULT1A3	16p11.2	L25275
201894_s_at	5.63	6.72	0.0185	2.76	3.22	0.0423	SSR1	6p24.3	NM_001920
203602_s_at	2.80	3.31	0.0191	2.11	2.28	0.0421	ZBTB17	1p36.2-p36.1	NM_003443
204017_at	0.92	1.33	0.0212	0.61	0.81	0.0204	KDELR3	22q13.1	NM_006855
632_at	4.09	4.87	0.0216	2.95	3.22	0.0469	GSK3A	19q13.2	L40027
217861_s_at	4.50	5.92	0.0234	3.92	4.76	0.00862	PREB	2p23.3	NM_013388
201082_s_at	4.96	6.43	0.0237	2.91	3.64	0.017	DCTN1	2p13	NM_004082
202722_s_at	2.60	3.05	0.0237	1.50	1.79	0.0135	GFPT1	2p13	NM_002056
200917_s_at	2.14	3.18	0.024	2.40	3.01	0.0168	SRPR	11q24.3	BG474541
212041_at	12.02	15.35	0.0248	8.48	10.10	0.0181	ATP6V0D1	16q22	AL566172
203648_at	4.87	5.81	0.0251	3.66	4.12	0.0332	TATDN2	3p25.3	NM_014760
65086_at	2.04	2.64	0.0284	3.05	3.39	0.0459	MGC3262	19p13.2	Z78349
216483_s_at	4.73	5.85	0.0313	3.90	4.56	0.0377	C19orf10	19p13.3	AC005339
204382_at	3.61	4.27	0.0323	3.50	3.94	0.0275	EBSP	17q25.1	NM_015654
216484_x_at	17.27	20.46	0.0341	10.56	11.45	0.0452	HDGF	1q21-q23	L24521
65635_at	3.31	3.80	0.0411	4.89	5.47	0.00597	FLJ21865	17q25.3	AL044097
200710_at	3.93	5.53	0.0441	2.96	4.02	0.000234	ACADVL	17p13-p11	NM_000018
214969_at	1.08	1.49	0.0449	0.95	1.12	0.0284	MAP3K9	14q24.3-q31	AF251442
203827_at	0.68	0.91	0.0469	0.32	0.37	0.00867	WIPI49	17q24.2	NM_017983
200825_s_at	15.90	20.22	0.0482	11.63	14.75	0.000148	HYOU1	11q23.1-q23.3	NM_006389

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Table 1 Continued.

	MAS			Affy					
Probe ID	Control	sXBP1	*p*-value	Control	sXBP1	*p*-value	Symbol	Map	Genbank
Down-regulated									
41386_i_at	7.91	4.71	0.000169	5.96	4.82	0.00104	JMJD3	17p13.1	AB002344
214317_x_at	71.11	32.66	0.000337	38.47	36.54	0.0339	RPS9	19q13.4	BE348997
201944_at	6.78	4.96	0.000353	3.12	2.54	0.0251	HEXB	5q13	NM_000521
212702_s_at	3.42	2.11	0.000548	2.66	2.20	0.0289	BICD2	9q22.31	N45111
202963_at	6.86	4.39	0.000589	3.94	3.42	0.0417	RFX5	1q21	AW027312
201487_at	9.06	5.18	0.000604	5.50	4.41	0.00736	CTSC	11q14.1-q14.3	NM_001814
202195_s_at	1.69	1.05	0.000607	0.76	0.60	0.0098	TMED5	1pter-q31.3	NM_016040
210361_s_at	1.89	1.30	0.000647	1.02	0.82	0.0185	ELF2	4q28	AF256223
218200_s_at	65.94	44.79	0.000799	35.38	31.07	0.0226	NDUFB2	7q34	NM_004546
204285_s_at	6.18	4.23	0.00109	3.99	3.07	0.0306	PMAIP1	18q21.32	AI857639
201339_s_at	5.39	4.06	0.00128	3.11	2.60	0.043	SCP2	1p32	NM_002979
204529_s_at	3.63	3.00	0.00137	2.12	1.76	0.00412	TOX	8q12.1	AI961231
220768_s_at	7.17	4.80	0.00142	5.06	3.73	0.0129	CSNK1G3	5q23	NM_004384
202278_s_at	3.89	3.01	0.00157	2.26	1.99	0.0224	SPTLC1	9q22.2	NM_006415
208456_s_at	2.23	1.77	0.00187	1.14	0.94	0.0447	RRAS2	11p15.2	NM_012250
204720_s_at	9.45	7.08	0.00194	7.19	5.40	0.0115	DNAJC6	1pter-q31.3	AV729634
202693_s_at	2.84	2.17	0.00228	1.75	1.38	0.000314	STK17A	7p12-p14	AW194730
201260_s_at	2.73	1.97	0.00237	1.66	1.44	0.00573	SYPL	7q22.3	NM_006754
208016_s_at	5.92	3.59	0.00291	3.12	2.65	0.0279	AGTR1	3q21-q25	NM_004835
203962_s_at	2.34	1.67	0.00308	1.20	0.99	0.0394	NEBL	10p12	NM_006393
221047_s_at	3.94	2.99	0.00313	2.71	2.34	0.0111	MARK1	1q41	NM_018650
204053_x_at	19.21	16.12	0.00371	10.87	9.48	0.00737	PTEN	10q23.3	U96180
212589_at	2.90	2.24	0.00406	2.17	1.73	0.0121	SCP2	1p32	AI753792
200750_s_at	70.41	54.89	0.00421	41.79	33.29	0.0162	RAN	12q24.3	AF054183
212560_at	1.50	1.07	0.00447	0.59	0.50	0.0397	C11orf32	–	AV728268
203613_s_at	20.82	16.41	0.00455	14.42	12.28	0.0141	NDUFB6	9p21.1	NM_002493
201580_s_at	5.41	4.42	0.00538	4.25	3.85	0.0101	DJ971N18.2	20p12	AL544094
211025_x_at	35.50	28.35	0.00538	22.64	20.13	0.00104	COX5B	2cen-q13	BC006229
221726_at	35.41	30.00	0.00571	22.23	19.44	0.0432	RPL22	1p36.3-p36.2	BE250348
212744_at	2.26	1.40	0.00573	1.20	1.02	0.0168	BBS4	15q22.3-q23	AI813772
201773_at	18.81	13.05	0.00575	11.61	9.20	0.0169	ADNP	20q13.13	NM_015339
221763_at	2.80	2.22	0.00636	1.42	1.25	0.0485	JMJD1C	10q21.3	AI694023
201142_at	7.81	6.62	0.00645	4.73	4.36	0.0365	EIF2S1	14q23.3	AA577698
218313_s_at	5.35	4.35	0.00665	3.25	2.84	0.0106	GALNT7	4q31.1	NM_017423
207467_x_at	3.42	2.58	0.00687	2.50	2.20	0.034	CAST	5q15-q21	NM_001750
201242_s_at	23.74	18.85	0.007	12.97	9.95	0.0346	ATP1B1	1q24	BC000006
212244_at	5.05	3.90	0.00726	3.63	3.22	0.0108	GRINL1A	15q22.1	AL050091
203493_s_at	1.74	1.15	0.00743	0.94	0.75	0.0339	PIG8	11q21	AL525206
216511_s_at	1.84	1.43	0.00768	0.86	0.74	0.0104	TCF7L2	10q25.3	AJ270770
222237_s_at	2.09	1.46	0.00777	1.39	1.22	0.00743	ZNF228	19q13.2	AC084239
202078_at	15.24	12.17	0.00912	12.44	10.14	0.0245	COPS3	17p11.2	NM_003653
200821_at	1.04	0.69	0.00924	0.30	0.27	0.0189	LAMP2	Xq24	NM_013995
212200_at	1.87	1.40	0.00959	0.69	0.60	0.0432	KIAA0692	12q24.33	AK025933
201385_at	38.11	24.54	0.0105	27.85	24.61	0.00227	DHX15	4p15.3	NM_001358
208923_at	23.50	20.92	0.0105	17.56	16.07	0.0391	CYFIP1	15q11	BC005097
202172_at	6.83	5.29	0.0106	4.28	3.75	0.0232	ZNF161	17q23.2	BG035116
219863_at	3.35	2.81	0.0107	1.68	1.47	0.00993	HERC5	4q22.1-q23	NM_016323
212476_at	1.45	1.02	0.0109	0.62	0.47	0.0182	CENTB2	3q29	D26069
202429_s_at	8.67	6.55	0.011	6.68	5.65	0.00231	PPP3CA	4q21-q24	AL353950
202143_s_at	12.21	10.42	0.0112	7.94	6.94	0.0425	COPS8	2q37.3	NM_006710
221208_s_at	3.20	2.26	0.0115	1.60	1.21	0.0267	FLJ23342	11q24.2	NM_024631

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Table 1 Continued.

	MAS			Affy					
Probe ID	Control	sXBP1	p-value	Control	sXBP1	p-value	Symbol	Map	Genbank
205934_at	1.23	0.86	0.0116	0.58	0.48	0.0134	PLCL1	2q33	NM_006226
201146_at	4.37	3.25	0.0119	2.74	2.37	0.0413	NFE2L2	2q31	NM_006164
203075_at	5.46	4.33	0.0119	2.68	2.14	0.00525	SMAD2	18q21.1	AW151617
207974_s_at	37.24	22.95	0.0119	24.85	18.32	0.0354	SKP1A	5q31	NM_006930
205329_s_at	3.72	2.83	0.0123	2.43	1.92	0.0453	SNX4	3q21.2	NM_003794
212459_x_at	3.46	3.00	0.0128	2.11	1.82	0.0403	SUCLG2	3p14.1	BF593940
200047_s_at	12.42	10.13	0.0129	7.67	6.10	0.0128	YY1	14q	NM_003403
202868_s_at	7.69	6.48	0.0129	4.87	4.34	0.0362	POP4	19q12	NM_006627
209300_s_at	3.25	2.65	0.0129	2.02	1.77	0.0367	NECAP1	12p13.31	BC002888
213129_s_at	16.10	11.45	0.013	12.59	9.27	0.0206	GCSH	16q23.2	AI970157
205308_at	4.30	3.40	0.0132	3.80	3.24	0.0162	CGI-62	8q21.11	NM_016010
210532_s_at	47.50	37.38	0.0132	27.18	23.61	0.0357	C14orf2	14q32.33	AF116639
219200_at	2.82	2.17	0.0132	2.08	1.58	0.0234	MGC5297	5p15.3-p15.2	NM_024091
207164_s_at	1.37	0.98	0.0136	0.47	0.42	0.00458	ZNF238ss	1q44-qter	NM_006352
218320_s_at	28.65	22.61	0.0137	15.89	14.33	0.0156	NDUFB11	Xp11.3	NM_019056
207654_x_at	9.87	7.83	0.0139	6.21	5.28	0.0434	DR1	1p22.1	NM_001938
203376_at	6.67	4.58	0.0143	4.19	3.73	0.0257	CDC40	6q21	BG528818
206038_s_at	1.05	0.80	0.0143	0.56	0.48	0.033	NR2C2	3p25	NM_003298
221933_at	2.53	1.62	0.0145	1.48	1.35	0.0118	NLGN4X	Xp22.32-p22.31	AI338338
203917_at	8.88	7.72	0.0146	6.32	5.41	0.00366	CXADR	21q21.1	NM_001338
212181_s_at	6.03	4.87	0.0158	4.25	3.88	0.00455	NUDT4	12q21	AF191654
218339_at	6.13	5.13	0.0158	4.39	3.69	0.00542	MRPL22	5q33.1-q33.3	NM_014180
204030_s_at	7.98	6.58	0.0161	3.59	3.01	0.00732	SCHIP1	3q25.32-q25.33	NM_014575
221916_at	13.80	8.15	0.0167	9.78	8.14	0.0427	NEFL	8p21	BF055311
211725_s_at	10.48	8.89	0.0177	8.37	7.05	0.00908	BID	22q11.1	BC005884
202457_s_at	7.30	6.42	0.0181	5.44	4.85	0.00333	PPP3CA	4q21-q24	AA911231
203216_s_at	1.68	1.26	0.0181	0.89	0.65	0.0103	MYO6	6q13	NM_004999
202393_s_at	1.67	1.19	0.0182	0.52	0.42	0.0359	KLF10	8q22.2	NM_005655
212230_at	3.39	2.58	0.0182	2.38	1.81	0.00389	PPAP2B	1pter-p22.1	AV725664
213249_at	5.69	4.50	0.0191	3.88	3.37	0.0112	FBXL7	5p15.1	AU145127
218209_s_at	7.83	5.46	0.0192	4.75	3.69	0.0365	P15RS	18q12.2	NM_018170
212229_s_at	10.54	8.77	0.0194	6.59	5.36	0.0317	FBXO21	12q24.22	AK001699
209206_at	1.73	1.44	0.0195	0.97	0.85	0.00979	SEC22L1	1q21.2-q21.3	AV701283
215091_s_at	26.02	22.60	0.0197	14.04	12.73	0.0242	GTF3A	13q12.3-q13.1	BE542815
219080_s_at	2.18	1.60	0.0198	1.40	1.26	0.00437	CTPS2	Xp22	NM_019857
200730_s_at	8.83	7.43	0.0201	5.20	4.39	0.0448	PTP4A1	6q12	BF576710
218379_at	4.04	3.10	0.0208	2.24	1.85	0.0222	RBM7	11q23.1-q23.2	NM_016090
200914_x_at	4.65	3.32	0.021	2.45	1.63	0.0499	KTN1	14q22.1	BF589024
201200_at	4.09	3.19	0.0213	2.92	2.21	0.00333	CREG1	1q24	NM_003851
210986_s_at	15.07	11.73	0.0216	8.30	6.32	0.006	TPM1	15q22.1	Z24727
203593_at	1.08	0.84	0.0222	0.27	0.24	0.0235	CD2AP	6p12	NM_012120
204435_at	4.32	2.87	0.0224	2.22	1.61	0.0325	NUPL1	13q12.13	NM_014778
208811_s_at	5.43	4.05	0.0224	5.34	4.22	0.0041	DNAJB6	7q36.3	AF080569
218549_s_at	3.58	2.58	0.0225	2.18	1.81	0.041	CGI-90	8q21.3	NM_016033
202352_s_at	15.61	13.62	0.0228	8.34	7.09	0.02	PSMD12	17q24.2	AI446530
213306_at	4.91	3.21	0.0231	3.82	3.30	0.0242	MPDZ	9p24-p22	AA917899
204634_at	1.69	1.34	0.0232	0.89	0.73	0.00523	NEK4	3p21.1	NM_003157
212455_at	16.88	15.23	0.0238	10.53	9.00	0.0159	YT521	4q13.2	N36997
208742_s_at	32.17	28.12	0.0248	18.20	16.95	0.0212	SAP18	13q12.11	U78303
218351_at	2.11	1.51	0.0255	2.02	1.67	0.00284	COMMD8	4p12	NM_017845
218055_s_at	3.40	2.53	0.0261	2.47	2.21	0.0189	WDR41	5q13.3	NM_018268
222273_at	0.79	0.59	0.0264	0.29	0.25	0.0201	–	–	AI419423

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Table 1 Continued.

	MAS			Affy					
Probe ID	Control	sXBP1	*p*-value	Control	sXBP1	*p*-value	Symbol	Map	Genbank
205176_s_at	12.25	10.34	0.0265	8.74	7.05	0.0127	ITGB3BP	1p31.3	NM_014288
214101_s_at	1.42	1.08	0.0272	0.64	0.51	0.0339	NPEPPS	17q21	BG153399
215321_at	0.79	0.55	0.0278	0.51	0.43	0.0489	RPIB9	7q21.12	AI825798
212446_s_at	4.39	3.38	0.0293	2.59	2.10	0.023	LASS6	2q24.3	AI658534
34764_at	2.26	1.87	0.0293	1.18	1.04	0.0433	LARS2	3p21.3	D21851
202202_s_at	1.03	0.79	0.0294	0.68	0.58	0.0211	LAMA4	6q21	NM_002290
201483_s_at	22.63	18.70	0.0296	15.06	14.25	0.0351	SUPT4H1	17q21-q23	BC002802
204353_s_at	5.39	3.94	0.0303	3.24	2.66	0.022	POT1	7q31.33	BC002923
202623_at	6.00	4.85	0.0305	3.62	2.96	0.026	C14orf11	14q13.1	NM_018453
200096_s_at	8.15	7.43	0.0315	5.37	4.57	0.0047	ATP6V0E	5q35.1	AI862255
204058_at	1.14	0.87	0.0315	0.47	0.40	0.0166	ME1	6q12	AL049699
208867_s_at	5.68	4.44	0.0316	4.50	4.14	0.0466	CSNK1A1	5q32	AF119911
216438_s_at	33.19	25.46	0.0324	12.32	10.00	0.0185	TMSB4X/// TMSL3	Xq21.3-q22/// 4q22.1	AL133228
221702_s_at	14.17	11.11	0.0324	10.85	9.74	0.0218	BLP2	15q26.3	AF353992
202651_at	7.45	6.02	0.0326	4.05	3.21	0.0406	LPGAT1	1p36.13-q42.3	NM_014873
204791_at	3.03	2.56	0.0327	1.25	1.00	0.0161	NR2C1	12q22	NM_003297
218698_at	8.47	4.93	0.0327	4.09	3.41	0.0188	MMRP19	11p13	NM_015957
203131_at	7.69	5.78	0.0342	4.17	3.63	0.0288	PDGFRA	4q11-q13	NM_006206
213883_s_at	3.60	2.44	0.0345	2.40	1.89	0.012	BBP	1p31.3	AA012917
202001_s_at	6.57	5.78	0.0352	5.90	4.95	0.0306	NDUFA6	22q13.2-q13.31	NM_002490
204872_at	7.07	5.02	0.0352	4.38	3.46	0.0399	TLE4	9q21.31	NM_007005
204049_s_at	2.40	1.64	0.0353	1.96	1.40	0.00427	PHACTR2	6q24.2	NM_014721
205168_at	6.30	5.51	0.0354	6.95	6.38	0.0366	DDR2	1q12-q23	NM_006182
214431_at	12.45	9.53	0.0362	7.96	6.25	0.00604	GMPS	3q24	NM_003875
209106_at	6.45	5.11	0.0367	4.17	3.59	0.026	NCOA1	2p23	BF576458
208693_s_at	42.22	35.22	0.037	26.59	23.45	0.0462	GARS	7p15	D30658
204246_s_at	18.25	15.77	0.0374	10.52	9.50	0.0373	DCTN3	9p13	NM_007234
202241_at	2.46	1.82	0.0375	1.78	1.52	0.0443	TRIB1	8q24.13	NM_025195
203372_s_at	2.85	2.36	0.0378	2.16	1.78	0.000286	SOCS2	12q	AB004903
213424_at	1.09	0.60	0.0378	0.31	0.26	0.0453	KIAA0895	7p14.1	AB020702
205656_at	5.11	4.29	0.0379	2.97	2.51	0.0418	PCDH17	13q21.1	NM_014459
212507_at	3.93	3.42	0.0387	3.04	2.50	0.0295	RW1	2q11.2	D87446
213513_x_at	11.41	9.51	0.0388	9.92	7.94	0.0105	ARPC2	2q36.1	BG034239
202003_s_at	9.22	7.11	0.0392	10.41	8.98	0.0405	ACAA2	18q21.1	NM_006111
204516_at	1.33	0.95	0.0393	0.99	0.83	0.0264	ATXN7	3p21.1-p12	BG390306
207845_s_at	1.61	1.17	0.0393	0.69	0.53	0.0215	ANAPC10	4q31	NM_014885
202342_s_at	3.40	2.89	0.0403	2.14	1.75	0.017	TRIM2	4q31.3	NM_015271
222011_s_at	4.09	3.53	0.0417	2.66	2.29	0.0374	TCP1	6q25.3-q26	BF224073
203137_at	10.39	8.68	0.0428	7.47	6.11	0.0393	WTAP	6q25-q27	NM_004906
57739_at	3.12	2.35	0.0453	2.44	2.05	0.0442	DND1	5q31.3	AI949010
207173_x_at	3.44	2.78	0.0454	1.81	1.47	0.0351	CDH11	16q22.1	D21254
204675_at	1.45	0.97	0.0456	0.70	0.54	0.0287	SRD5A1	5p15	NM_001047
201498_at	7.73	6.40	0.0457	5.52	4.72	0.0305	USP7	16p13.3	AI160440
218718_at	2.42	2.06	0.0457	1.35	1.05	0.0194	PDGFC	4q32	NM_016205
218611_at	3.05	2.46	0.046	1.52	1.27	0.0478	IER5	1q25.3	NM_016545
205429_s_at	2.63	2.10	0.0461	1.71	1.37	0.0267	MPP6	7p15	NM_016447
203621_at	26.17	21.42	0.0464	16.97	15.33	0.0359	NDUFB5	3q26.33	NM_002492
202786_at	8.08	6.72	0.0465	6.27	5.09	0.0141	STK39	2q24.3	NM_013233
212077_at	11.01	8.69	0.048	6.88	5.91	0.0262	CALD1	7q33	AL583520
218125_s_at	2.31	1.81	0.0482	2.59	2.18	7.82E-05	FLJ10853	8p21.1	NM_018246
217266_at	4.04	2.86	0.0496	2.58	2.08	0.00307	RPL15/// LOC136321	3p24.2/// 7q33	Z97353

The genes were sorted according to the *p*-value calculated from the normalized data by MAS 5.0.

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DNA containing nucleotides −147 to −96. We incubated 20 fmol biotin-labeled double-stranded DNA with 4 μg nuclear extract in EMSA buffer in a final volume of 20 μL. Binding competition was performed using a 200-fold excess of unlabeled oligonucleotides. A supershift assay was performed using 2 μg anti-c-myc antibody (A-14; Santa Cruz Biotechnology, Santa Cruz, CA, USA.). EMSAs were also performed using 6 μg nuclear extracts of *XBP1+/+* MEFs and *XBP1−/−* MEFs.

Results

WFS1 is induced by overexpression of XBP1

To identify the target genes of XBP1, we performed gene expression analysis using DNA microarray in SH-SY5Y cells transfected with sXBP1-expressing vector ($n = 5$) and control vector ($n = 5$). Putative XBP1-regulated genes were selected by the following criteria: (1) called as present in all samples and (2) parametric test showed significant difference ($p < 0.05$) between two groups by both normalization methods (MAS5.0 and RMA). As a result, 50 up-regulated genes and 154 down-regulated genes were selected among 9849 genes that were present. This list included *Erdj4* (*DNAJB9*), which has been shown to be regulated mainly by XBP1 (Lee *et al.* 2003; Kanemoto *et al.* 2005). Among the genes listed, the highest *p*-value was observed for *WFS1* (NM_006005) (Table 1), and we focused on the effects of XBP1 on *WFS1* expression in the following analyses. The difference in *WFS1* mRNA levels between sXBP1 overexpression and control was confirmed by real-time quantitative PCR (control vector, mean ± SEM 0.00772 ± 0.00023; sXBP1-expressing vector, 0.0127 ± 0.00089; $p = 0.00063$, *t*-test) (Fig. 1). In a similar experiment ($n = 3$), we independently confirmed that the overexpression of sXBP1 induced the expression of *WFS1* (control vector, 0.00990 ± 0.000475; sXBP1-expressing vector, 0.01675 ± 0.000936; $p = 0.00284$, *t*-test).

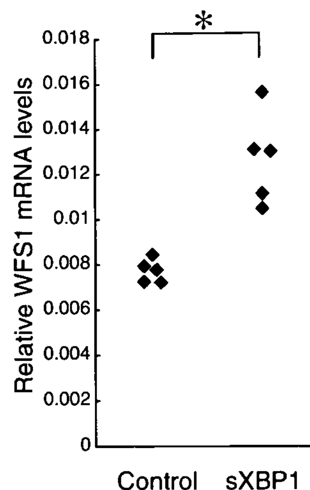

Fig. 1 Confirmation of DNA microarray results by quantitative real-time PCR. Induction of *WFS1* by sXBP1 overexpression in SH-SY5Y cells was confirmed. The mRNA levels were normalized using *GAPDH* as the standard. *$p = 0.00063$ (*t*-test).

Identification of an ERSE-like sequence in the promoter region

We next examined the promoter region of the *WFS1* gene and found several elements that might be responsible for its induction by XBP1 overexpression. A sequence that resembles nutrient-sensing response element-1 (NSRE-1; GGATGAAA) was found within nucleotides −384 to −377 (from the start codon) and NSRE-2 (GTTACA) was within nucleotides −340 to −335. An ERSE-like sequence (CGAGGCGC-ACCGTGATTGGCGG; similar to the consensus sequence of ERSE CCAAT-N(9)-CCACG) was identified within nucleotides −140 to −122. Interestingly, a 11-bp sequence of the ERSE-like sequence (GTGATTGGCGG) appeared downstream of the ERSE-like sequence (− 109 to −99). Comparison of several mammalian genomes revealed that the ERSE-like sequence and the 11-bp repeated sequence on the *WFS1* promoter are well conserved across species (Fig. 2).

Search for ERSE and its variants in other regulated genes

We examined whether other genes altered by XBP1 contain ERSE and its variants. Although 18 of 204 genes had ERSEs in their genomic region spanning from the −9-kb upstream region of exon 1 to the −2-kb downstream region of the last exon (Table 2), only two genes (*WFS1* and *GCSH*) had ERSE-like sequences in their upstream region near the first exon. *GCSH* was one of the genes down-regulated by XBP1. Thus, *WFS1* seems to be the only gene that is both induced by XBP1 and contains ERSE-like sequence in its upstream region.

ERSE-like sequence is critical for activation by XBP1

We characterized experimentally the promoter of *WFS1*. We used a luciferase assay system with or without the NSRE-1-, NSRE-2- and ERSE-like sequences, as well as the 11-bp repeated sequence. We transfected SH-SY5Y cells with reporter constructs containing the promoter fragment of interest, a reference vector, and an sXBP1-expressing vector or control vector. Co-transfection of sXBP1-expressing vector significantly enhanced the promoter activity only when an ERSE-like sequence was contained in the reporter plasmid (Fig. 3). The results clearly demonstrate that an ERSE-like sequence is critical for the response to sXBP1 overexpression. The 11-bp sequence did not seem to have a major role in XBP1-dependent induction of *WFS1*.

XBP1 does not bind to the ERSE-like sequence directly

We then examined, using EMSA, whether or not XBP1 protein binds directly to the ERSE-like sequence of the *WFS1* promoter region. We incubated a biotin-labeled, double-stranded oligonucleotide probe containing nucleotides −147

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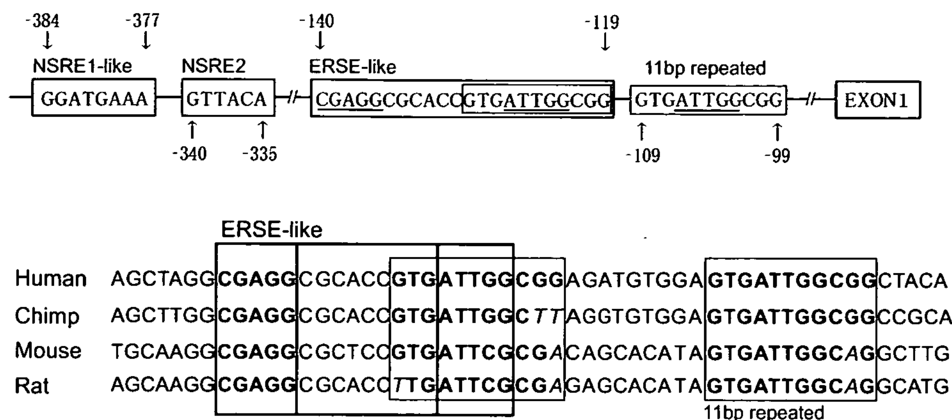

Fig. 2 Promoter region of *WFS1*. *WFS1* has an ERSE-like element on its promoter (upper panel), which is highly conserved among mammals (lower panel). In addition to the ERSE-like sequence, a well-conserved 11-bp repeated sequence, which is the same as part of the ERSE-like element, was also found near the ERSE-like sequence.

to – 96 (which includes an ERSE-like sequence) with a nuclear extract of cells transfected with c-myc-tagged sXBP1-expressing vector. When electrophoresed, a band shift appeared (Fig. 4a, lane 2). This band shift was almost completely inhibited by competition with a 200-fold excess of unlabeled oligonucleotides (lane 3). The supershift was not observed when anti-c-myc antibody was added (lane 4), although an immunoprecipitation assay showed a reaction of the antibody to myc-tagged XBP1 under the same condition. Thus, XBP1 may not bind directly to the ERSE-like sequence. We further performed an EMSA using *XBP1–/–* MEFs. A band shift was also observed in *XBP1–/–* MEFs (Fig. 4b, lane 4). However, the amount of shifted band from *XBP1–/–* MEFs was less than that from *XBP1+/+* MEFs. This suggests that some transcription factor regulated by XBP1 binds to the ERSE-like sequence in the *WFS1* promoter.

Table 2 ERSE and its variants found in the genes altered by overexpression of XBP1

Motif	Probe ID	Symbol	Sequence	Location	
ERSE-like	Up-regulated				
	212041_at	ATP6V0D1	CCAATATGGCAAAACCTCG	5′-Flanking	from -7210 to -7192
	202908_at	WFS1	CGAGGCGCACCGTGATTGG	5′-Flanking	from -140 to -122
	212052_s_at	KIAA0676	CGAGGTTTCACCATATTGG	Intron7	
	Down-regulated				
	215321_at	RPIB9	CGAGGTACACCCTCATTGG	Intron2	
	203962_s_at	NEBL	CCAATTGTTTCTAACCTCG	Intron4	
	212702_s_at	BICD2	CCAATCACAATGTGCCTCG	Intron1	
	213129_s_at	GCSH	CGAGGCAGGGTCTGATTGG	5′-Flanking	from -106 to -88
	212476_at	CENTB2	CCAATGTGGTGAAACCTCG	Intron18	
	216511_s_at	TCF7L2	CGAGGCTGGAGTGCATTGG	Intron5	
	203216_s_at	MYO6	CCAATATGGTGAAACCTCG	Intron19	
ERSE	Up-regulated				
	201082_s_at	DCTN1	CGTGGTGCTCTTAAATTGG	Intron6	
	Down-regulated				
	215321_at	RPIB9	CGTGGATATAGTAAATTGG	5′-Flanking	from -8530 to -8512
	204872_at	TLE4	CCAATACCCCTACTCCACG	Exon12	
	219200_at	MGC5297	CGTGGAGCCTTTGGATTGG	5′-Flanking	from -6727 to -6709
	202786_at	STK39	CCAATACCCGGCAGCCACG	Intron14	
	204720_s_at	DNAJC6	CGTGGTTTTGAGAAATTGG	Intron1	
ERSE-CHOP	Down-regulated				
	204872_at	TLE4	CGTGCAGAAATGGTATTGG	Intron7	
	205176_s_at	ITGB3BP	CGTGCCTTATTGACATTGG	5′-Flanking	from -7763 to -7745
	221933_at	NLGN4X	CCAATTCCCTCAAAGCACG	Intron3	

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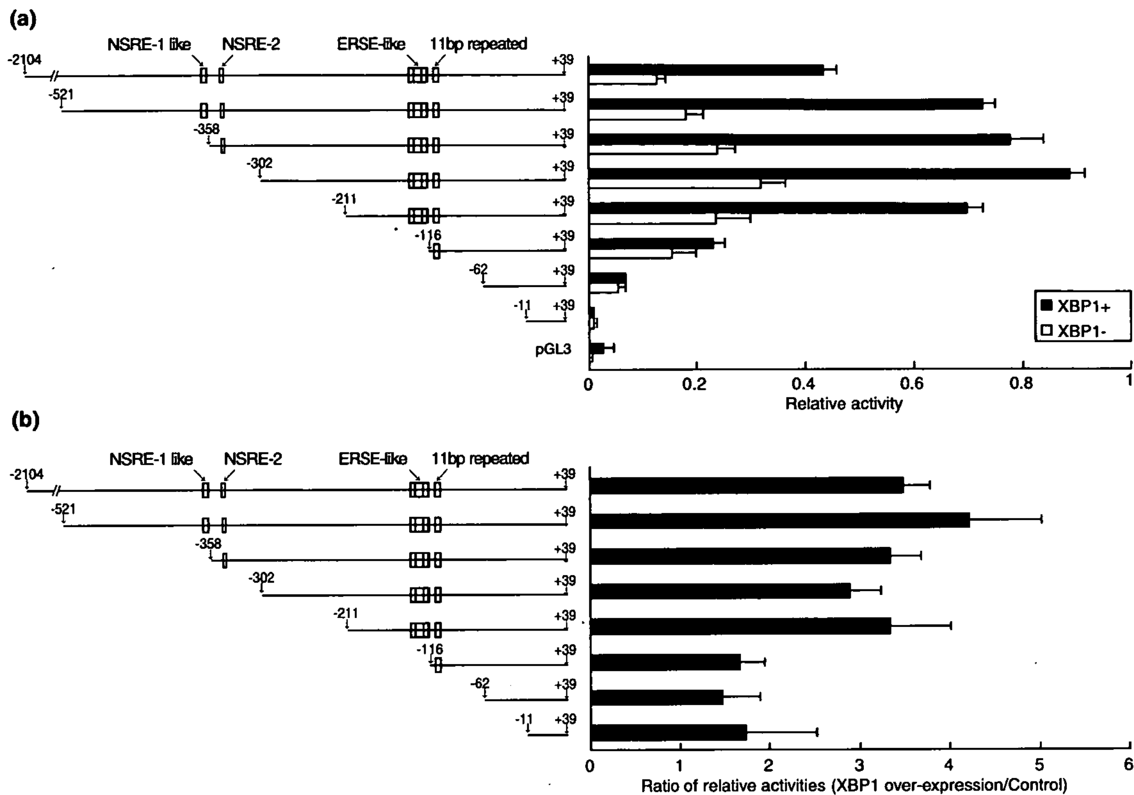

Fig. 3 Promoter assay. (a) Mean ± SEM basal relative activities (ratio of firefly luciferase to that of *Renilla*) with or without sXBP1-expressing vector co-transfection. (b) The ratio (XBP1 Overexpression/Control) of the relative promoter activities of the upper data. The effect of co-transfection of the sXBP1-expressing vector was lost when the ERSE-like sequence was missing. Each of these experiments was repeated three times.

Discussion

We demonstrated in this report that WFS1 expression is regulated by XBP1 through an ERSE-like sequence, not by binding directly.

The ERSE is observed in ER chaperones (*GRP78*, *GRP94*, *calreticulin*), as well as *CHOP* and *XBP1* (Yoshida *et al.* 1998, 2001; Ubeda and Habener 2000). Although the consensus sequence of ERSE is CCAAT-N9-CCACG, its variant, CCAAT-N9-GCACG, is also reported to be functional in the *CHOP* gene. The ERSE-like sequence of *WFS1* may also be another variant of ERSE. Recently, *WFS1* was reported to be induced by ER stressors such as thapsigargin and dithiothreitol in pancreatic islets (Yamaguchi *et al.* 2004; Ueda *et al.* 2005). This previously reported induction via transcriptional activation might be through this ERSE sequence.

In addition to the ERSE of *WFS1*, a 11-bp sequence including CCAAT near the ERSE is also highly conserved in mammals. Although we could not observe the effect of sXBP1-dependent promoter activity, it cannot be ruled out that this element enhances the promoter activity of the ERSE-like sequence.

Several ERSE-binding proteins have been reported, such as ATF6, NF-Y, YY1 and TFII-I (Yoshida *et al.* 1998, 2000; Li *et al.* 2000; Parker *et al.* 2001). Of these, expression of the gene for YY1 was significantly altered, but it was down-regulated. None of these was found to be up-regulated by XBP1. XBP1 itself is also known to bind to ERSE in the presence of NF-Y (Yoshida *et al.* 1998; Yamamoto *et al.* 2004). However, anti-c-myc antibody did not cause a supershift in the EMSA, and nuclear extracts from *XBP1–/–* MEFs could also cause a band shift. These findings suggest that XBP1 does not directly bind to the ERSE-like sequence of *WFS1*. The amount of shifted band was smaller in *XBP1–/–* MEFs, suggesting that some transcription factor regulated by XBP1 binds to the ERSE-like sequence on the *WFS1* promoter.

WFS1 was initially identified as a causative gene for Wolfram disease (Inoue *et al.* 1998; Strom *et al.* 1998; Hardy *et al.* 1999). Wolfram disease (OMIM in NCBI website 222300) is a rare autosomal-recessive disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness. Swift and colleagues reported that about 60% of altered individuals have some mental disturbance, such as severe depression, psychosis or organic brain syndrome, as

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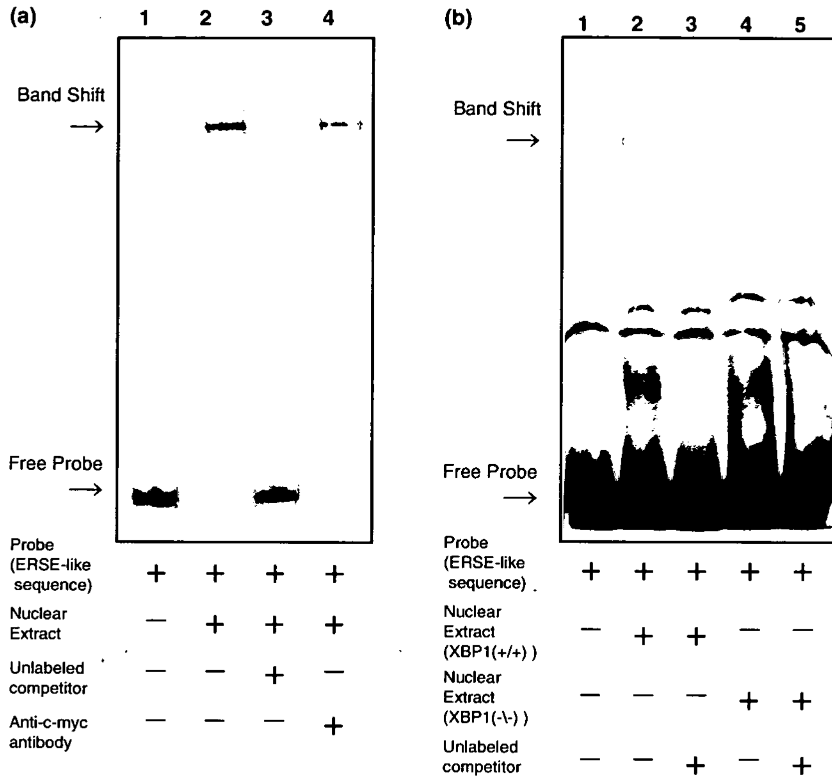

Fig. 4 XBP1 does not bind to the ERSE-like sequence directly. (a) In an EMSA, a band shift was observed when probes and nuclear extracts were incubated together (lane 2) and this band shift was inhibited when a 200-fold excess of unlabeled probes was used (lane 3). We did not observe a supershift when using anti-c-myc antibody (lane 4). (b) In XBP1−/− MEFs, a band shift was observed (lane 4), although the amount of shifted band was less than that seen in XBP1+/+ MEFs (lane 2).

well as impulsive verbal and physical aggression (Swift *et al.* 1990). They further reported that even the heterozygotes who do not have Wolfram disease are 26 times more likely than non-carriers to have a psychiatric hospitalization, mainly because of severe depression (Swift and Swift 2000). WFS1 protein predominantly localizes to ER (Takeda *et al.* 2001), and induces cation channel activity on the ER membrane (Osman *et al.* 2003). However, its molecular function is still not well characterized. Our results, as well as those in two other recent reports (Yamaguchi *et al.* 2004; Ueda *et al.* 2005), clearly indicate that *WFS1* is involved in the UPR pathway.

In addition to *WFS1*, we suggest that the gene encoding GCSH is another candidate for XBP1-mediated regulation through an ERSE-like sequence. *GCSH* has an ERSE-like sequence in its upstream region near the first exon, where the known ERSEs, as well as the ERSE-like sequence of *WFS1*, are typically found. However, GCSH expression was down-regulated when XBP1 was overexpressed. GCSH is a component of the enzyme system for cleavage of glycine (OMIM 238330), and a defect in GCSH is implicated in glycine encephalopathy (OMIM 605899). A link between this gene and the ER stress response has not yet been reported. Further studies are required to clarify the functional role of this motif.

In summary, we screened for target genes of XBP1 in neuroblastoma cells and identified *WFS1* as a strong candidate. An as-yet-unknown transcription factor is thought to mediate this induction. Further studies are needed to clarify further details of ER stress signaling in neuronal cells.

Acknowledgements

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References

Bruning J. C., Winnay J., Cheatham B. and Kahn C. R. (1997) Differential signaling by insulin receptor substrate 1 (IRS-1) and IRS-2 in IRS-1-deficient cells. *Mol. Cell. Biol.* **17**, 1513–1521.

Hardy C., Khanim F., Torres R., Scott-Brown M., Seller A., Poulton J., Collier D., Kirk J., Polymeropoulos M., Latif F. and Barrett T. (1999) Clinical and molecular genetic analysis of 19 Wolfram syndrome kindreds demonstrating a wide spectrum of mutations in WFS1. *Am. J. Hum. Genet.* **65**, 1279–1290.

Inoue H., Tanizawa Y., Wasson J. *et al.* (1998) A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). *Nat. Genet.* **20**, 143–148.

Irizarry R. A., Hobbs B., Collin F., Beazer-Barclay Y. D., Antonellis K. J., Scherf U. and Speed T. P. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264.

Iwamoto K., Kakiuchi C., Bundo M., Ikeda K. and Kato T. (2004) Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol. Psychiatry* **9**, 406–416.

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Kakiuchi C., Iwamoto K., Ishiwata M. *et al.* (2003) Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. *Nat. Genet.* **35**, 171–175.

Kakiuchi C., Ishiwata M., Kametani M., Nelson C., Iwamoto K. and Kato T. (2005) Quantitative analysis of mitochondrial DNA deletions in the brains of patients with bipolar disorder and schizophrenia. *Int. J. Neuropsychopharmacol.* **8**, 515–522.

Kanemoto S., Kondo S., Ogata M., Murakami T., Urano F. and Imaizumi K. (2005) XBP1 activates the transcription of its target genes via an ACGT core sequence under ER stress. *Biochem. Biophys. Res. Commun.* **331**, 1146–1153.

Lee A. H., Iwakoshi N. N. and Glimcher L. H. (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell. Biol.* **23**, 7448–7459.

Li M., Baumeister P., Roy B., Phan T., Foti D., Luo S. and Lee A. S. (2000) ATF6 as a transcription activator of the endoplasmic reticulum stress element: thapsigargin stress-induced changes and synergistic interactions with NF-Y and YY1. *Mol. Cell. Biol.* **20**, 5096–5106.

Osman A. A., Saito M., Makepeace C., Permutt M. A., Schlesinger P. and Mueckler M. (2003) Wolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. *J. Biol. Chem.* **278**, 52 755–52 762.

Parker R., Phan T., Baumeister P., Roy B., Cheriyath V., Roy A. L. and Lee A. S. (2001) Identification of TFII-I as the endoplasmic reticulum stress response element binding factor ERSF: its autoregulation by stress and interaction with ATF6. *Mol. Cell. Biol.* **21**, 3220–3233.

Reimold A. M., Etkin A., Clauss I. *et al.* (2000) An essential role in liver development for transcription factor XBP-1. *Genes Dev.* **14**, 152–157.

Schroder M. and Kaufman R. J. (2005) ER stress and the unfolded protein response. *Mutat. Res.* **569**, 29–63.

Sevinsky J. R., Whalen A. M. and Ahn N. G. (2004) Extracellular signal-regulated kinase induces the megakaryocyte GPIIb/CD41 gene through MafB/Kreisler. *Mol. Cell. Biol.* **24**, 4534–4545.

Shim J., Umemura T., Nothstein E. and Rongo C. (2004) The unfolded protein response regulates glutamate receptor export from the endoplasmic reticulum. *Mol. Biol. Cell* **15**, 4818–4828.

Strom T. M., Hortnagel K., Hofmann S., Gekeler F., Scharfe C., Rabl W., Gerbitz K. D. and Meitinger T. (1998) Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. *Hum. Mol. Genet.* **7**, 2021–2028.

Swift M. and Swift R. G. (2000) Psychiatric disorders and mutations at the Wolfram syndrome locus. *Biol. Psychiatry* **47**, 787–793.

Swift R. G., Sadler D. B. and Swift M. (1990) Psychiatric findings in Wolfram syndrome homozygotes. *Lancet* **336**, 667–669.

Takeda K., Inoue H., Tanizawa Y., Matsuzaki Y., Oba J., Watanabe Y., Shinoda K. and Oka Y. (2001) WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. *Hum. Mol. Genet.* **10**, 477–484.

Todaro G. J. and Green H. (1963) Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *J. Cell Biol.* **17**, 299–313.

Ubeda M. and Habener J. F. (2000) *CHOP* gene expression in response to endoplasmic-reticular stress requires NFY interaction with different domains of a conserved DNA-binding element. *Nucleic Acids Res.* **28**, 4987–4997.

Ueda K., Kawano J., Takeda K. *et al.* (2005) Endoplasmic reticulum stress induces *Wfs1* gene expression in pancreatic beta-cells via transcriptional activation. *Eur. J. Endocrinol.* **153**, 167–176.

Vandenberghe W., Nicoll R. A. and Bredt D. S. (2005) Interaction with the unfolded protein response reveals a role for stargazin in biosynthetic AMPA receptor transport. *J. Neurosci.* **25**, 1095–1102.

Yamaguchi S., Ishihara H., Tamura A., Yamada T., Takahashi R., Takei D., Katagiri H. and Oka Y. (2004) Endoplasmic reticulum stress and N-glycosylation modulate expression of WFS1 protein. *Biochem. Biophys. Res. Commun.* **325**, 250–256.

Yamamoto K., Yoshida H., Kokame K., Kaufman R. J. and Mori K. (2004) Differential contributions of ATF6 and XBP1 to the activation of endoplasmic reticulum stress-responsive cis-acting elements ERSE, UPRE and ERSE-II. *J. Biochem. (Tokyo)* **136**, 343–350.

Yoshida H. (2004) Molecular biology of the ER stress response. *Seikagaku* **76**, 617–630.

Yoshida H., Haze K., Yanagi H., Yura T. and Mori K. (1998) Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. *J. Biol. Chem.* **273**, 33 741–33 749.

Yoshida H., Okada T., Haze K., Yanagi H., Yura T., Negishi M. and Mori K. (2000) ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the *cis*-acting element responsible for the mammalian unfolded protein response. *Mol. Cell. Biol.* **20**, 6755–6767.

Yoshida H., Okada T., Haze K., Yanagi H., Yura T., Negishi M. and Mori K. (2001) Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response. *Mol. Cell. Biol.* **21**, 1239–1248.

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Comprehensive Gene Expression Analysis in Bipolar Disorder

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Objective: To review recent findings by DNA microarray in bipolar disorder (BD).

Method: A literature search was performed.

Results: Comprehensive gene expression analysis in the brain, peripheral blood cells, and olfactory neuroepithelium would be a promising strategy for the research of BD. To date, alterations in glutamate receptors (GR), mitochondria-related genes, chaperone genes, oligodendrocyte genes, and markers of gamma amino butyric acidergic (GABAergic) neurons in postmortem brains are replicated by several different strategies. However, alterations in mitochondria-related genes are associated with agonal factors, sample pH, and effects of drugs. Analysis of blood cells showed altered endoplasmic reticulum stress pathway and other molecular cascades. Analysis of olfactory epithelium showed altered expression of genes associated with apoptosis.

Conclusions: These findings warrant that comprehensive gene expression analysis by DNA microarray will be useful to identify the molecular cascades responsible for BD.

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Clinical Implications

- Gene expression analysis in postmortem brains, blood cells, and olfactory neuroepithelium will be useful to develop biomarkers for BD.
- Alterations in GRs, mitochondria-related genes, chaperone genes, oligodendrocyte genes, and GABAergic markers are reported in postmortem brains.
- Alterations in endoplasmic reticulum stress and apoptosis pathways were suggested by the analysis of blood cells and neuroepithelium.

Limitations

- Many findings have not yet been well replicated.
- These findings need to be verified in larger samples.
- Alterations in mitochondria-related genes in postmortem brains are associated with agonal factors, sample pH, and effects of drugs.

Key Words: *bipolar disorder, glutamate receptors, mitochondria, chaperones, oligodendrocyte, GABA, brain, lymphoblastoid cells*

In spite of extensive studies, pathophysiology of BD is still enigmatic. The role of genetic factors is well established from twin studies, but the causative genes or genetic risk factors have not been well established.[1] The other well-studied area of biological research is neuroimaging. Those studies suggested that the volume of several brain areas, such as anterior cingulate, amygdala, and hippocampus, are altered in BD.[2] However, the results are not always consistent with each other. The most established finding is that there is increased incidence of subcortical hyperintensity detected by MRI, although such a finding is not specific to BD because this is frequently seen in healthy, elderly people. The third important area of research would be postmortem brain studies.[3] Before the SMRI[4] started its activity to provide brain bank samples to anyone with an adequate research plan, the number of postmortem brain studies of BD patients was quite small. Since SMRI started to provide the samples, the number of studies dramatically increased and the postmortem brain study became a hot research area. Another area of study involved obtaining peripheral blood samples. One replicated finding obtained from such studies is altered basal or agonist-stimulated calcium levels in blood cells derived from patients with BD.[5] In addition to those studies involving patients' samples, studies focusing on the pharmacology of mood stabilizers are also widely performed. To date, a number of hypotheses to account for the action mechanisms of mood stabilizers have been suggested and are still controversial.

Abbreviations used in this article

BD	bipolar disorder
ER	endoplasmic reticulum
GABA	gamma amino butyric acid
GABAergic	gamma amino butyric acidergic
GAD	glutamic acid decarboxylase
GR	glutamate receptor
GRIK1	glutamate receptor kainate 1
HSPF1	heat shock protein 40
LARS2	mitochondrial leucyl-tRNA synthetase
MRI	magnetic resonance imaging
NPY	neuropeptide Y
PCR	polymerase chain reaction
PKC	protein kinase C
RT-PCR	reverse transcribed quantative polymerase chain reaction
SMRI	Stanley Medical Research Institute
SNP	single nucleotide polymorphism
SST	somatostatin
TGF-1β1	transforming growth factor-beta 1

In this situation, the recent development of comprehensive gene expression analysis using DNA microarray is expected to be a powerful tool to identify molecular pathophysiology of BD (see Figure 1). Since Bezchlibnyk et al[6] first used DNA microarray for the study of BD, this technology has been applied to postmortem brains, peripheral leukocytes, transformed lymphoblastoid cells, and biopsied olfactory neuroepithelium derived from patients with BD.

In this review, studies focusing on molecular cascades related to pathophysiologic mechanisms of BD are summarized (see Table 1). After the postmortem brain studies are summarized by subcategorizing the findings into several sections, studies of peripheral blood cells and neuroepitherium are introduced. Finally, limitations and future directions are presented. Detailed methodological issues regarding the platforms used for the DNA microarray analysis and the method of data analysis were discussed elsewhere.[7]

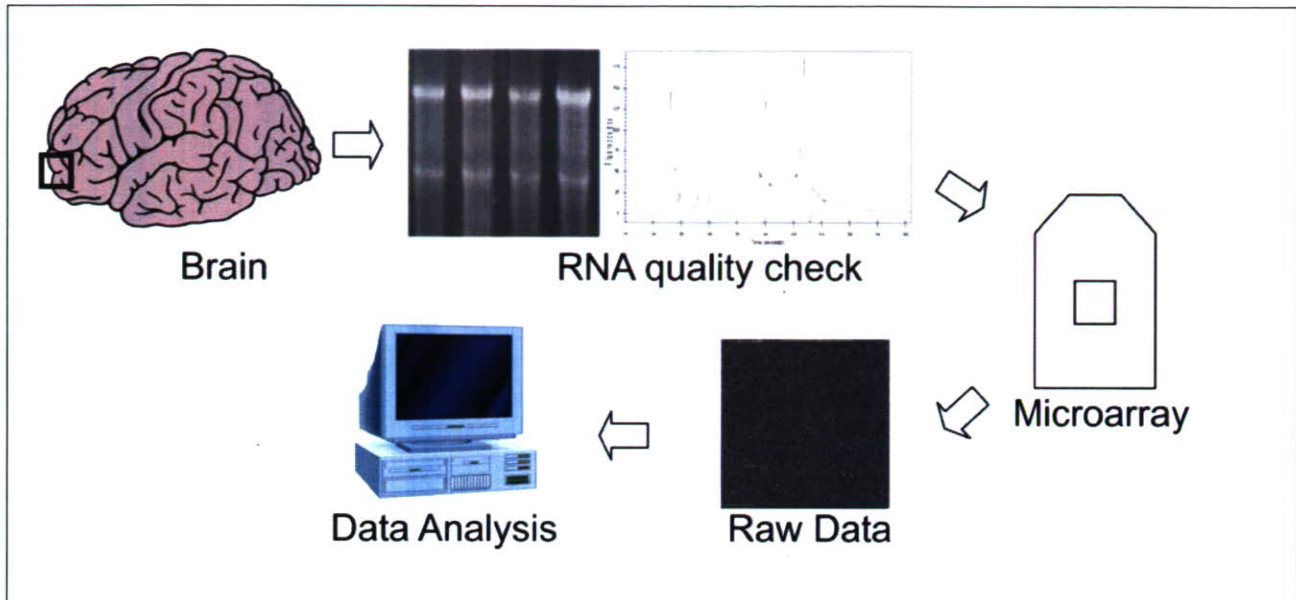
Postmortem Brain Studies

Initial Studies

Sun and colleagues[8] used the serial analysis of gene expression method to identify the differentially expressed genes in the postmortem brains of a patient with BD in comparison with an age- and sex-matched control subject. They found upregulation of serotonin transporter and NF-κB2 genes and confirmed these findings in a case–control study in the brains of 19 patients with BD and 15 controls. The upregulation of serotonin transporter mRNA in BD may be compatible with a recent finding showing increase of serotonin transporter binding potential in vivo measured by positron emission tomography.[9] A recent study showed increased levels of NF-κB protein in lymphocytes obtained from 15 patients with bipolar depression, compared with 25 controls,[10] which may be in accordance with the initial finding in postmortem brains.

Bezchlibnyk et al[6] used a cDNA microarray carrying about 1200 genes to search for genes relevant to biochemical process of BD. They mixed the RNA extracted from postmortem brain samples obtained from 10 patients with BD or 10 controls obtained from Stanley Foundation Neuropathology Consortium and analyzed by a set of cDNA microarray. They reported differential expression of 24 genes, and focused on 5 candidate genes. They validated the finding of decreased TGF-1β1 by analyzing individual samples by RT-PCR method. The results showed statistically significant decrease of TGF-1β1 expression. TGF-1β1 is a multifunctional cytokine involved in various functions such as cell growth and differentiation, and thus a possible role in BD was suggested. Reduced TGF-1β1 level was also reported in plasma of 70 patients with mania, compared with 96 controls.[11] It was rather higher in patients with schizophrenia,[12] and higher

Figure 1 The methodology of DNA microarray analysis. RNA is extracted from brain samples. The quality of RNA is checked by gel electrophoresis and agilent bioanalyzer. RNA samples are reverse-transcribed using oligo dT primer. From the cDNA, biotynated cRNAs are produced by in vitro transcription. The cRNA sample is used for GeneChip analysis (Affymetrix). The raw data are analyzed after normalization is applied.



scores of Hamilton Depression Rating Scale were associated with lower levels.[13] These findings support the initial finding and suggest a possible role of TGF-1β1 as a biomarker of BD. However, owing to its multiple functions, its specificity and clinical significance as a biomarker may be challenged.

Expression of PDLIM5

Iwamoto et al[14] performed comprehensive gene expression analysis of the postmortem brains obtained from the same brain resource as Bezchlibnyk et al, but used one oligonucleotide microarray per sample. They found altered expression of 53 genes. These included downregulation of neurotransmission-related genes such as calcium channel P/Q type alpha 1A, serotonin 2C receptor, metabotropic GR (GRM1), ionotropic GRIK1, and increased stress response-related genes. Among the differentially expressed genes found in the postmortem brains, altered expression of HSPF1 and LIM (PDLIM5) was also seen in lymphoblastoid cells derived from patients with BD. HSPF1 was increased both in the brain and lymphoblastoid cells, while PDLIM5 was increased in the brain but decreased in the lymphoblastoid cells. PDLIM5 encodes an adaptor protein linking PKC and N-type calcium channel. Since alterations of both calcium signalling and PKC activity have been reported in BD, they further pursued the role of this gene in BD. Decreased expression of PDLIM5 in lymphoblastoid cells was further verified in an extended sample of bipolar I disorder, as well as those with bipolar II disorder and schizophrenia.[15] Genetic association analysis showed association with BD, while the association with schizophrenia is not consistent in 2 studies.[16,17] More recently, reduced expression of PDLIM5 was found to be related to depressive state.[18] These combined findings suggest the role of PDLIM5 expression may be a biomarker for mental disorders.

Chaperones and Stress Response Genes

Jurata et al[19] also performed comprehensive gene expression analysis in the postmortem brains of patients with BD and controls. They used 2 platforms, oligonucleotide microarray (Affymetrix Hu133A) and cDNA microarray (Agilent Human 1), to compare the efficiency of these platforms to detect differentially expressed genes. First, they used parietal cortex samples of 1 bipolar patient and 1 control. They subsequently examined 8 prefrontal cortex samples each of patients with BD and controls. They concluded that sensitivity to detect the differential expression was higher in oligonucleotide array than in cDNA microarray. They reported 47 genes showing differential expression using both platforms. There was no overlap with the findings by Bezchlibnyk et al.[6] However, upregulation of genes of chaperones or stress response proteins, such as GRP94 (gp96) and interferon-induced transmembrane protein 2, is similar to the finding by Iwamoto et al,[14] who reported upregulation of several chaperone or stress response genes such as HSPF1 and

Table 1 Summary of major findings

Findings	Postmortem brain	Peripheral blood/neuroepithelium
NF-κB	BP↑[8]	BP↑(protein)[10]
TGF-1β1	BP↑[6]	Mania↓[11]
		Depression↓[13]
		Schizophrenia↑[12]
PDLIM5	BP↑[14,17]	BPI, BPII↓[14,15]
		Depression↓[18]
Chaperones	BP↑[14,19]	HSPF1, BPI, BPII↓[14,15]
	Depression↑[20]	
Mitochondria-related genes	BP↓[14,21,25,26]	BPI[32]
NDUFV2	BP↑[28,29]	BP↓[31,32]
LARS2	BP↑[27]	—
Ubiquitin pathway	BP↓[34]	—
GABA neuron markers	BP, NPY↓[53]	—
	BP, SST, GAD1↓[21]	
	BP, SST↑[29]	
GABA and glutamate receptors	BP[41]	—
	BP↓GRIK1[14,41]	
Oligodendrocyte genes	BP↓[43]	
ER stress pathway	—	BP↓[56]
APOBEC3B, etc	—	BP[61]
MAX	—	BP↑[62]
Apoptosis-related genes	—	BP↑[63]

interferon induced transmembrane protein 3. Upregulation of HSPF1 was also found in lymphoblastoid cell lines in patients with both bipolar I[14] and bipolar II disorders.[15] Increased expression of endoplasmic reticulum chaperons such as GRP78, GRP94, and calreticulin is also reported in patients with major depression who died by suicide.[20]

Mitochondria-Related Genes

Konradi et al[21] performed gene expression analysis in the hippocampal samples of 9 patients with BD and 10 controls, as well as 8 patients with schizophrenia, obtained from Harvard Brain Bank, using oligonucleotide microarray. They found that mitochondria-related genes were globally downregulated in BD. They found that genes related to mitochondrial oxidative phosphorylation or ubiquitin proteasome pathway. They discussed that the effect of drugs cannot explain this finding because patients with schizophrenia taking similar drugs did not show such marked global downregulation of mitochondria-related genes. They interpreted that this finding reflects mitochondrial dysfunction in BD.

Global downregulation of mitochondria-related genes had also been observed in postmortem brains samples with low pH or agonal factors such as prolonged hypoxia and ischemia.[22,23] Indeed, sample pH was significantly lower in BD group in the study of Konradi et al.[21] Thus the finding in BD may be explained by those factors. Iwamoto et al[24] performed DNA microarray analysis in the Stanley Microarray Collection samples. Whereas the global downregulation of mitochondria-related genes in BD reported by Konradi et al[21] was replicated, lower sample pH was associated with downregulation of mitochondria-related genes. In addition, several classes of psychotropic drugs, such as antipsychotics, valproate, and antidepressants, were found to be associated with reduced expression of mitochondria-related genes. In

drug-free patients, mitochondria-related genes were instead upregulated.

Vawter and colleagues[25] as well as Sun et al[26] also replicated the global downregulation of mitochondria-related genes in BD. Vawter et al[25] minutely analyzed the interaction between disease and confounding factors to observe the gene expression patterns in postmortem brains. Whereas they classified the genes affected by disease and (or) agonal-pH factors, they were prudent enough to avoid proposing any particular genes as specific to BD. They also found that copy number of mtDNA tended to be increased in the patients with BD. Sun et al[26] replicated the finding of global downregulation of mitochondria-related genes and effects of pH, but proposed that reduced pH itself might be a reflection of mitochondrial dysfunction in BD.

Munakata et al[27] reanalyzed the previously reported DNA microarray data focusing on mitochondrial genes. They found that LARS2 was upregulated in BD. Because a well characterized mitochondrial DNA 3243 A>G mutation on mitochondrial leucyl tRNA is reported to impair the amioacylation of tRNA catalyzed by LARS2, they hypothesized that this finding might be compensatory upregulation by the presence of the mtDNA 3243 mutation. They verified that LARS2 was indeed upregulated in the cybrids carrying 3243G mutation and, using the protein nucleic acid-clamped PCR method, they searched for this mutation in the postmortem brains. They found that 3 patients, 2 with BD and 1 with schizophrenia, showed relatively high level of 3243 mutation, not only in the brain but also in the liver. This suggested that accumulation of mtDNA mutation in the brain might play a part in the pathophysiology of BD.

While many mitochondria-related genes were reported to be downregulated, possibly reflecting low sample pH, increased expression of NDUFV2 encoding 24kDa subunit of complex I in parietotemporal or frontal cortices was reported by 2 groups.[28,29] This is compatible with upregulation of NDUFV2 in the rat depression model of learned helplessness.[30] On the other hand, reduced NDUFV2 level was found in lymphoblastoid cells of BD patients.[31] Reduced NDUFV2 level was correlated with downregulation of other mitochondria-related genes in lymphoblastoid cells.[32] Although this was initially attributed to promoter polymorphisms,[31,33] the genetic association was not replicated in extended samples.[29]

Ubiquitin Pathway

Ryan et al[34] examined the prefrontal and orbitofrontal cortices samples from the Stanley Microarray Collection and performed comprehensive gene expression analysis. No significant alteration was found in the prefrontal cortex, while ubiquitin pathway is dowregulated and G-protein coupled receptors were upregulated in orbitofrontal cortex.

GABA Neuron Markers

In the data by Konradi et al,[21] downregulation of GAD67 and SST, both of which are markers of GABAergic neurons, was also reported. Downregulation of SST and GAD67 was replicated in our analysis (Iwamoto et al, unpublished finding). Somatostatin concentrations in cerebrospinal fluid in patients with BD showed conflicting results: decrease,[35] no change,[36] or increase.[37] These findings may be in accordance with reduced GAD67 positive GABAergic neurons in BD[38,39] or decreased GABA concentration measured by in vivo proton magnetic resonance spectroscopy.[40] Conversely, Nakatani et al[29] used the samples from an Australian brain bank to perform gene expression analysis and found the differential expression of 8 genes (RAP1GA1, SST, HLA-DRA, KATNB1, PURA, NDUFV2, STAR, and PAFAH1B3), including the upregulation of SST. Although the observed change of SST was in the opposite direction to previous studies, their subsequent genetic association study showed the association between SNP markers of SST and BD.

GABA and Glutamate Receptors

Choudary et al[41] reported that 2 GABA receptor genes (GABRA5 and GABBR1) were upregulated in the dorsolateral prefrontal cortex. Dysfunction of subpopulation of GABAergic interneurons and compensatory upregulation of GABAergic receptors on pyramidal neurons has been suggested as a pathophysiological mechanism of schizophrenia.[42] These observations may reflect the pathophysiology shared by these 2 mental disorders.

Three GR genes (GRIA1, GRIA3, and GRM3) were also shown to be upregulated in the dorsolateral prefrontal cortex and (or) anterior cingulate cortex. On the other hand, downregulation of GRIK1 encoding kainate type GR found in this study is in accordance with other reports.[14,29]

Comparison With Schizophrenia

Tkachev et al[43] performed gene expression analysis in the postmortem brains of patients with schizophrenia and found global downregulation of oligodendrocyte-related genes such as myelin-associated glycoprotein and myelin oligodendrocyte glycoprotein. Similar findings were reported by the other investigators in various brain regions.[44–50] They used RT-PCR and found that downregulation of oligodendrocyte-related genes was also seen in BD. We did not detect such downregulation in the initial sample set; however, we could replicate it in the second sample set (Iwamoto et al, unpublished finding).

While Konradi and colleagues[21] reported global dowregulation of mitochondrial genes in BD but not in schizophrenia, Alter et al[51] dissected hippocampal pyramidal

neurons and found that mitochondria-related genes were downregulated in schizophrenia but not in BD.

Downregulation of the GABAergic neuron marker genes and (or) loss of GABAergic interneurons, were also reported in schizophrenia.[52] In one initial study, Kuromitsu et al[53] used oligonucleotide microarray for gene expression analysis of pooled RNA samples from patients with schizophrenia and controls. They found that NPY was downregulated in the brains of patients with schizophrenia. Downregulation of NPY in the prefrontal cortex was also confirmed in BD by RT-PCR. NPY is one of several peptides expressed in GABAergic interneurons. This finding is compatible with a previously reported finding.[54]

These findings suggest that downregulation of GABAergic interneuron-related genes, mitochondria-related genes, and oligodendrocyte-related genes may be common to schizophrenia and BD, although there are some inconsistencies. However, it is also possible that these apparent alterations might reflect the effects of confounding factors such as agonal-pH factors, medication, and cause of death.

When all confounding factors are controlled, the number of samples and the power of analysis decreases. Thus it is a matter of debate about how these factors should be controlled.[7]

Public Database

As described above, SMRI played a significant role in the postmortem brain studies of BD. After this brain bank started to distribute the brain samples to researchers, the number of papers on postmortem brain studies in BD markedly increased. Although intellectual property rights belonged to the SMRI when these brain bank samples were used, these data were not used for commercial purpose but were made open to the public.[55] This database will further facilitate the research of BD. However, the list of differentially expressed genes in the database tend to be different from the original report, reflecting the different methods for data analysis, exclusion of confounding factors, and so on. This might potentially cause confusion among the researchers who are not familiar with the DNA microarray studies using postmortem brains.

Summary of Findings

As written above, altered expression of chaperone and stress response genes, mitochondria-related genes, GABAergic neuron markers such as SST and GAD1, GRIK1 encoding kinate type GR, and oligodendrocyte-related genes were replicated in more than one study. Several expression changes detected in postmortem brains, such as NF-κB, TGF-1β1, PDLIM5, HSPF1, and NDUFV2, were also detected in peripheral blood cells and thus might be used as biomarkers for BD.

Peripheral Blood Cells and Olfactory Neuroepithelium

Monozygotic Twins Discordant for BD

Kakiuchi et al[56] used oligonucleotide microarray for the analysis of lymphoblastoid cell lines derived from 2 pairs of monozygotic twins discordant for BD and 1 pair of control twins. Among the genes commonly downregulated in these 2 pairs of twins, XBP1 and HSPA5 (GRP78) were related to ER stress response, and they further focused on this pathway. ER stress response was attenuated in the lymphoblastoid cells derived from patients with BD in a small case–control study. They identified a SNP, -116 C/G, which impairs XBP1-induced XBP1 expression, owing to loss of XBP1 binding site on its promoter. This SNP was associated with BD in the original report, but subsequent studies did not support this association.[57,58] More recently, So and colleagues[59] also reported that XBP1 induction by ER stress was attenuated in the lymphoblastoid cells derived from patients with BD, but this was not solely explained by XBP1 polymorphism. By searching for the target genes of XBP1 in neuroblastoma cells using DNA microarray, Kakiuchi et al[60] found that WFS1, the causative gene for Wolfram syndrome, is induced by XBP1 through ER stress response element on its promoter. Wolfram syndrome is known to comorbid with mood disorders. This finding supports the role of XBP1-WFS1 pathway in BD.

Search of Peripheral Blood for Biomarkers

Tsuang et al[61] analyzed the gene expression patterns in peripheral blood leukocytes derived from patients with BD and reported that APOBEC3B, ADSS, ATM, CLC, CTBP1, DATF1, CXCL1, and S100A9 would become biomarkers to distinguish the patients from those with schizophrenia or controls. Middleton et al[62] also performed gene expression analysis of peripheral blood leukocytes. By comparing the patients with their healthy sib pairs, upregulation of myc-associated factor X was found. For other 248 genes altered in BD, they discussed the significance of the finding in relation to the genetic linkage loci, and suggested that chromosome 6q showed both linkage and gene expression alteration signals.

Olfactory Neuroepithelium

Considering the difficulties in managing confounding factors in the study of postmortem brain studies, use of olfactory neuroepithelium would be a promising alternative, in spite of its invasiveness. McCurdy et al[63] performed gene expression analysis in biopsied olfactory epithelium derived from patients with BD. They found that cultures from BD patients showed high ratio of cell death, and alteration of apoptosis-related genes were detected by their original oligonucleotide microarray. Genes associated with intracellular inositol signalling were also altered.

Summary of Findings

In summary, endoplasmic reticulum stress pathway was implicated in the pathophysiology of BD based on the findings of discordant twins. Several genes found by gene expression analysis of peripheral blood cells were proposed as biomarkers of BD. A study in olfactory neuroepithelium showed enhanced apoptotic pathway in BD.

Discussion

As described above, data of gene expression analysis in postmortem brains cannot be free from artifacts, owing to premortem or antemortem changes. Such changes may be linked with diagnosis, because the cause of death is different, and pathophysiology of the illness itself may affect these processes. In addition, effect of drugs always confounds the results. Due to these difficulties, the findings obtained by the gene expression analysis using postmortem brains are not conclusive on their own.

Conversely, the analysis of peripheral blood cells also has problems because it is not the target organ of mental disorder and many important genes expressed in the brain are not expressed in peripheral blood cells. Thus only abnormalities of basic metabolism could be assessed in peripheral blood cells. In addition, effects of drugs are difficult to exclude in clinical settings. It is also difficult to distinguish the effect of trait-dependent alteration from state-dependent alteration. Use of lymphoblastoid cell lines would be one way to control these problems.

Use of olfactory neuroepithelium biopsy samples may be a promising alternative. However, because of its invasiveness, such research has been done in only limited institutions.

The only way to obtain conclusive results would be to verify the findings obtained in postmortem brains by multiple approaches, such as analysis of peripheral blood, genetics, animal models, and so on. When the data obtained from these different approaches are compared, methodological consideration is essential because each research strategy has characteristic artifacts, which should be carefully controlled.

In spite of the methodological problems, several findings, such as alterations in GRs, PDLIM5, mitochondrial dysfunction, impaired endoplasmic reticulum stress response, and alterations in GABAergic neurons, are validated by several different strategies. Before concluding that these pathways are important for the pathophysiology of BD, we should verify that these findings are caused by disease process rather than effects of confounding factors using animal models. To clarify the causal relation, behavioural consequence of animal models having abnormalities in these pathways should be examined. If the roles of these cascades are confirmed by those studies, these findings should be tested as peripheral biomarkers. Further studies would clarify the etiology of BD and provide clinically useful biomarkers.

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References

1. Kato T. Molecular genetics of bipolar disorder and depression. Psychiatry Clin Neurosci. 2007;61:1–17.
2. Friedman JN, Hurley RA, Taber KH. Bipolar disorder: imaging state versus trait. J Neuropsychiatry Clin Neurosci. 2006;18:296–301.
3. Dean B. The neurobiology of bipolar disorder: findings using human postmortem central nervous system tissue. Aust N Z J Psychiatry. 2004;38:135–140.
4. Torrey EF, Webster M, Knable M, et al. The Stanley foundation brain collection and neuropathology consortium. Schizophr Res. 2000;44:151–155.
5. Yamawaki S, Kagaya A, Tawara Y, et al. Intracellular calcium signaling systems in the pathophysiology of affective disorders. Life Sci. 1998;62:1665–1670.
6. Bezchlibnyk YB, Wang JF, McQueen GM, et al. Gene expression differences in bipolar disorder revealed by cDNA array analysis of post-mortem frontal cortex. J Neurochem. 2001;79:826–834.
7. Iwamoto K, Kato T. Gene expression profiling in schizophrenia and related mental disorders. Neuroscientist. 2006;12:349–361.
8. Sun Y, Zhang L, Johnston NL, et al. Serial analysis of gene expression in the frontal cortex of patients with bipolar disorder. Br J Psychiatry Suppl. 2001;41:s137–141.
9. Cannon DM, Ichise M, Fromm SJ, et al. Serotonin transporter binding in bipolar disorder assessed using [11C]DASB and positron emission tomography. Biol Psychiatry. 2006;60:207–217.
10. Spiliotaki M, Salpeas V, Malitas P, et al. Altered glucocorticoid receptor signaling cascade in lymphocytes of bipolar disorder patients. Psychoneuroendocrinology. 2006;31:748–760.
11. Kim YK, Myint AM, Lee BH, et al. T-helper types 1, 2, and 3 cytokine interactions in symptomatic manic patients. Psychiatry Res. 2004;129:267–272.
12. Kim YK, Myint AM, Lee BH, et al. Th1, Th2 and Th3 cytokine alteration in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28:1129–1134.
13. Myint AM, Leonard BE, Steinbusch HW, et al. Th1, Th2, and Th3 cytokine alterations in major depression. J Affect Disord. 2005;88:167–173.
14. Iwamoto K, Kakiuchi C, Bundo M, et al. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. Mol Psychiatry. 2004;9:406–416.
15. Iwamoto K, Bundo M, Washizuka S, et al. Expression of HSPF1 and LIM in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia. J Hum Genet. 2004;49:227–231.
16. Horiuchi Y, Arai M, Niizato K, et al. A polymorphism in the PDLIM5 gene associated with gene expression and schizophrenia. Biol Psychiatry. 2006;59:434–439.
17. Kato T, Iwayama Y, Kakiuchi C, et al. Gene expression and association analyses of LIM (PDLIM5) in bipolar disorder and schizophrenia. Mol Psychiatry. 2005;10:1045–1055.
18. Iga J, Ueno S, Yamauchi K, et al. Gene expression and association analysis of LIM (PDLIM5) in major depression. Neurosci Lett. 2006;400:203–207.
19. Jurata LW, Bukhman YV, Charles V, et al. Comparison of microarray-based mRNA profiling technologies for identification of psychiatric disease and drug signatures. J Neurosci Methods. 2004;138:173–188.
20. Bown C, Wang JF, MacQueen G, et al. Increased temporal cortex ER stress proteins in depressed subjects who died by suicide. Neuropsychopharmacology. 2000;22:327–332.
21. Konradi C, Eaton M, MacDonald ML, et al. Molecular evidence for mitochondrial dysfunction in bipolar disorder. Arch Gen Psychiatry. 2004;61:300–308.
22. Li JZ, Vawter MP, Walsh DM, et al. Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. Hum Mol Genet. 2004;13:609–616.
23. Tomita H, Vawter MP, Walsh DM, et al. Effect of agonal and postmortem factors on gene expression profile: quality control in microarray analyses of postmortem human brain. Biol Psychiatry. 2004;55:346–352.
24. Iwamoto K, Bundo M, Kato T. Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet. 2005;14:241–253.

25. Vawter MP, Tomita H, Meng F, et al. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. Mol Psychiatry. 2006;11:615, 663–679.
26. Sun X, Wang JF, Tseng M, et al. Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. J Psychiatry Neurosci. 2006;31:189–196.
27. Munakata K, Iwamoto K, Bundo M, et al. Mitochondrial DNA 3243A>G mutation and increased expression of LARS2 gene in the brains of patients with bipolar disorder and schizophrenia. Biol Psychiatry. 2005;57:525–532.
28. Karry R, Klein E, Ben Shachar D. Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry. 2004;55:676–684.
29. Nakatani N, Hattori E, Ohnishi T, et al. Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: relevance to neuronal network perturbation. Hum Mol Genet. 2006;15:1949–1962.
30. Nakatani N, Aburatani H, Nishimura K, et al. Comprehensive expression analysis of a rat depression model. Pharmacogenomics J. 2004;4:114–126.
31. Washizuka S, Kakiuchi C, Mori K, et al. Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder. Am J Med Genet B Neuropsychiatr Genet. 2003;120:72–78.
32. Washizuka S, Kakiuchi C, Mori K, et al. Expression of mitochondria-related genes in lymphoblastoid cells from patients with bipolar disorder. Bipolar Disord. 2005;7:146–152.
33. Washizuka S, Iwamoto K, Kazuno AA, et al. Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder in Japanese and the National Institute of Mental Health pedigrees. Biol Psychiatry. 2004;56:483–489.
34. Ryan MM, Lockstone HE, Huffaker SJ, et al. Gene expression analysis of bipolar disorder reveals downregulation of the ubiquitin cycle and alterations in synaptic genes. Mol Psychiatry. 2006;11:965–978.
35. Rubinow DR. Cerebrospinal fluid somatostatin and psychiatric illness. Biol Psychiatry. 1986;21:341–365.
36. Berrettini WH, Nurnberger JI Jr, Zerbe RL, et al. CSF neuropeptides in euthymic bipolar patients and controls. Br J Psychiatry. 1987;150:208–212.
37. Sharma RP, Bissette G, Janicak PG, et al. Elevation of CSF somatostatin concentrations in mania. Am J Psychiatry. 1995;152:1807–1809.
38. Woo TU, Walsh JP, Benes FM. Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. Arch Gen Psychiatry. 2004;61:649–657.
39. Benes FM, Vincent SL, Todtenkopf M. The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. Biol Psychiatry. 2001;50:395–406.
40. Bhagwagar Z, Wylezinska M, Jezzard P, et al. Reduction in occipital cortex gamma-aminobutyric acid concentrations in medication-free recovered unipolar depressed and bipolar subjects. Biol Psychiatry. 2007;61(6):806–812.
41. Choudary PV, Molnar M, Evans SJ, et al. Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. Proc Natl Acad Sci U S A. 2005;102:15653–15658.
42. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci. 2005;6:312–324.
43. Tkachev D, Mimmack ML, Ryan MM, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet. 2003;362:798–805.
44. Hakak Y, Walker JR, Li C, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A. 2001;98:4746–4751.
45. Sugai T, Kawamura M, Iritani S, et al. Prefrontal abnormality of schizophrenia revealed by DNA microarray: impact on glial and neurotrophic gene expression. Ann N Y Acad Sci. 2004;1025:84–91.
46. Iwamoto K, Bundo M, Yamada K, et al. DNA methylation status of SOX10 correlates with its downregulation and oligodendrocyte dysfunction in schizophrenia. J Neurosci. 2005;25:5376–5381.
47. Aston C, Jiang L, Sokolov BP. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. Mol Psychiatry. 2005;10:309–322.
48. Katsel P, Davis KL, Haroutunian V. Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study. Schizophr Res. 2005;79:157–173.
49. Aberg K, Saetre P, Jareborg N, et al. Human QKI, a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia. Proc Natl Acad Sci U S A 2006;103:7482–7487.
50. Dracheva S, Davis KL, Chin B, et al. Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. Neurobiol Dis. 2006;21:531–540.
51. Altar CA, Jurata LW, Charles V, et al. Deficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. Biol Psychiatry. 2005;58:85–96.
52. Pongrac J, Middleton FA, Lewis DA, et al. Gene expression profiling with DNA microarrays: advancing our understanding of psychiatric disorders. Neurochem Res. 2002;27:1049–1063.
53. Kuromitsu J, Yokoi A, Kawai T, et al. Reduced neuropeptide Y mRNA levels in the frontal cortex of people with schizophrenia and bipolar disorder. Brain Res Gene Expr Patterns. 2001;1:17–21.
54. Caberlotto L, Hurd YL. Reduced neuropeptide Y mRNA expression in the prefrontal cortex of subjects with bipolar disorder. Neuroreport. 1999;10:1747–1750.
55. Higgs BW, Elashoff M, Richman S, et al. An online database for brain disease research. BMC Genomics. 2006;7:70.
56. Kakiuchi C, Iwamoto K, Ishiwata M, et al. Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. Nat Genet. 2003;35:171–175.
57. Hou SJ, Yen FC, Cheng CY, et al. X-box binding protein 1 (XBP1) C--116G polymorphisms in bipolar disorders and age of onset. Neurosci Lett. 2004;367:232–234.
58. Cichon S, Buervenich S, Kirov G, et al. Lack of support for a genetic association of the XBP1 promoter polymorphism with bipolar disorder in probands of European origin. Nat Genet. 2004;36:783–784; author reply 784–785.
59. So J, Hun X, Warsh JJ, et al. Changes in endoplasmic reticulum stress responsivity in bipolar disorder. Biol Psychiatry. 2006;59:227S.
60. Kakiuchi C, Ishiwata M, Hayashi A, et al. XBP1 induces WFS1 through an endoplasmic reticulum stress response element-like motif in SH-SY5Y cells. J Neurochem. 2006;97:545–555.
61. Tsuang MT, Nossova N, Yager T, et al. Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report. Am J Med Genet B Neuropsychiatr Genet. 2005;133:1–5.
62. Middleton FA, Pato CN, Gentile KL, et al. Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches. Am J Med Genet B Neuropsychiatr Genet. 2005;136:12–25.
63. McCurdy RD, Feron F, Perry C, et al. Cell cycle alterations in biopsied olfactory neuroepithelium in schizophrenia and bipolar I disorder using cell culture and gene expression analyses. Schizophr Res. 2006;82:163–173.

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Résumé : Une analyse exhaustive de l'expression génétique dans le trouble bipolaire

Objectif : Examiner les résultats récents d'un jeu ordonné de microéchantillons d'ADN dans le trouble bipolaire (TB).

Méthode : Une recherche de la documentation a été exécutée.

Résultats : Une analyse exhaustive de l'expression génétique dans le cerveau, des globules de sang périphérique, et du neuroépithéliome olfactif serait une stratégie prometteuse pour la recherche sur le TB. Jusqu'ici, les modifications des récepteurs de glutamate (RG), des gènes liés aux mitochondries, des gènes chaperons, des gènes oligodendrocytes, et des marqueurs des neurones d'acide gamma-aminobutyrique (GABAergiques) dans les cerveaux postmortem sont reproduites par plusieurs stratégies différentes. Cependant, les modifications des gènes liés aux mitochondries sont associées avec des facteurs agonaux, un échantillon de pH, et les effets des médicaments. L'analyse des globules sanguins a montré une modification de la voie de stress du réticulum endoplasmique et d'autres cascades moléculaires. L'analyse de l'épithéliome olfactif a montré une expression modifiée des gènes associés à l'apoptose.

Conclusions : Ces résultats garantissent que l'analyse exhaustive de l'expression génétique par jeu ordonné de microéchantillons d'ADN sera utile pour identifier les cascades moléculaires responsables du TB.

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SPECIAL FEATURE

Frontiers in Epigenetics Medicine

Epigenetics in mood disorders

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Abstract Depression develops as an interaction between stress and an individual's vulnerability to stress. The effect of early life stress and a gene–environment interaction may play a role in the development of stress vulnerability as a risk factor for depression. The epigenetic regulation of the promoter of the glucocorticoid receptor gene has been suggested as a molecular basis of such stress vulnerability. It has also been suggested that antidepressive treatment, such as antidepressant medication and electroconvulsive therapy, may be mediated by histone modification on the promoter of the brain-derived neurotrophic factor gene. Clinical genetic studies in bipolar disorder suggest the role of genomic imprinting, although no direct molecular evidence of this has been reported. The role of DNA methylation in mood regulation is indicated by the antimanic effect of valproate, a histone deacetylase inhibitor, and the antidepressive effect of *S*-adenosyl methionine, a methyl donor in DNA methylation. Studies of postmortem brains of patients have implicated altered DNA methylation of the promoter region of membrane-bound catechol-*O*-methyltransferase in bipolar disorder. An altered DNA methylation status of PPIEL (peptidylprolyl isomerase E-like) was found in a pair of monozygotic twins discordant for bipolar disorder. Hypomethylation of PPIEL was also found in patients with bipolar II disorder in a case control analysis. These fragmentary findings suggest the possible role of epigenetics in mood disorders. Further studies of epigenetics in mood disorders are warranted.

Keywords Biopolar disorder · DNA methylation · Environment · Epigenetics · Mood disorders · Stress vulnerability

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Introduction

It is clear that both genes and the environment confer risk for mood disorders. A relative recent development in the field of biological psychiatry has been the focus on attempts to understand functional outcomes of the additive and combinatorial effects of genes and the environment at the molecular level [1]. As such, the interplay between a relatively fixed genome and an often variable environment involves epigenetic factors.

Epigenetic changes are long-lasting modifications in gene function that do not involve changes in gene sequences. Recent evidence suggests that these changes may occur in both dividing and nondividing cells [2–5] and may be transmitted intergenerationally [6, 7]. Epigenetic mechanisms involve modifications of the functional unit of the genome, the nucleosome, which is composed primarily of an octamer of pairs of H2A, H2B, H3, and H4 histones, around which is wrapped a 147-bp segment of DNA [8]. This configuration allows for the regulation of transcription through the control of access to the gene. Although many epigenetic modifications influence gene regulation, in the context of molecular psychiatric analysis, the most prevalently studied modifications to date are DNA methylation of CpG dinucleotides and acetylation and methylation at the N-terminal tails of histones. DNA is methylated by the transfer of a methyl group from *S*-adenosyl methionine

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