Letter to the Editor

Bioinformatics Analysis Raises Candidate Genes in Blood for Early Screening of Parkinson's Disease*

ZHANG Yi[1,△], YAO Li[2,△], LIU Wei[3], LI Wei[4], TIAN Chan[5], WANG Zhao Yang[3,#], and LIU Di[4,#]

Parkinson's disease (PD) is a typical degenerative disease, which is characterized by the most obvious symptoms of movement dysfunction, including shaking, rigidity, slowness of movement and difficulty in walking and gait. This disease can not be clearly identified through laboratory tests at present, thus application of high-throughput technique in studying the expression profiles of PD helps to find the genetic markers for its early diagnosis. Studies on expression profiles of neurodegenerative diseases have revealed the novel genes and pathways involved in the progress of illness. In this study, the expression profiles of PD in blood were compared, showing that 181 differentially expressed genes (DEG) exhibit a similar expression trend both in patients and in normal controls. These genes are enriched significantly in some biological processes, including development, response to drugs, and DNA-dependent regulation of transcription, etc, highlighting that the genetic markers can be used in early diagnosis of PD.

Parkinson's disease (PD) is a degenerative disorder, which mainly affects motor neurons[1]. The most obvious symptoms in its early stage are shaking, rigidity, slowness of movement and difficulty in walking and gait. Behavioral and thinking problems may occur with development of dementia in its advanced stage[1]. PD usually disturbs people over 50 years of age. The lifetime risk of PD is 1.5% and 1.5-fold higher in men than in women[2]. The pathology of PD is characterized by the accumulation of alpha-synuclein into inclusions called Lewy bodies in neurons, and from insufficient formation and activity of dopamine produced in certain neurons within parts of the midbrain. The cause of PD is complex[3]. PD can be diagnosed according to the medical history and neurological examinations. PD can not be clearly identified through laboratory tests at present, although brain scanning can sometimes exclude disorders that give rise to similar symptoms. Usually, the diagnosis of PD can be established when Lewy bodies are found in the midbrain. As a matter of fact, some authorities recommend that periodic diagnosis should be conducted, as the progress of the illness over time may reveal that it is actually not PD[4].

A common strategy for studying neurodegenerative diseases is to the high-throughput technique for screening the gene expression profiles in diseased and neurologically healthy control brain samples. Our recent studies on prion disease showed that genes are differentially expressed in disease samples. Moreover, s Alzheimer's disease and oxidative phosphorylation, etc, may play an essential role in the pathogenesis of PD[5-6].

However, it is hardly to obtain brain tissue samples from healthy people for early diagnosis of neurodegenerative diseases. Therefore, finding markers in blood will greatly contribute to the early diagnosis of neurodegenerative diseases. Scherzer et al.[7] showed that 8 genes display a significant difference in 66 patients. In the present study, both cortex and blood expression profiles of PD in blood were analyzed in order to find more candidate genes in blood for its early diagnosis. Particularly, we are looking for those genes with similar expression trends in patients versus normal controls in both brain and blood.

Expression profiles of both cortex and blood samples of PD patients were obtained from the ExpressArray database. Twenty-seven microarray

doi: 10.3967/ bes2014.074

1. Cell Engineering Research Center, State Key Laboratory of Cancer Biology, Fourth Military Medical University, Xi'an 710032, Shaanxi, China; 2. Department of Pathology, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, Shaanxi, China; 3. The School of Acupuncture and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China; 4. Network Information Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China; 5. State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases (Hangzhou, China, 310003), National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

data from PD patients' cortex and 26 controls (E-GEOD-20168)[8], and 50 microarray data from PD patients' blood and 23 controls (E-GEOD-6613)[7] were used in the analysis of expression profiles. The microarrays from different platforms were normalized using the software dChip, and the normalized signal values were to represent the expression levels of individual genes in blood. The normalization was performed using an 'invariant set' automatically selected by dChip and then followed by a 'Running median' method. The normalization processes made each microarray's scatterplot comparable. After normalization, 8 475 differentially expressed genes (DEG) shared by all microarrays were used in further analysis and the DEG with a ratio greater than 1.2 were filtered. A total of 1 280 or 2 101 DEG were detected in brain cortex or blood in PD patients and filtered, showing 96 genes were up-regulated and 85 genes were down-regulated in both samples.

The ontology (GO) of 181 genes with a similar expression trend in both cortex and blood were analyzed, showing that the up-regulated genes affect 322 terms of biological processes (BP) while the down-regulated genes affect 161 terms of BP. The top three-affected BP were the development, response to drugs, and DNA-dependent regulation of transcription (Table 1) with 11, 4, and 9 genes involved. The P- and Q-values were very low, implying a significant significance in BP. It should note that the 'response to drug', which was significantly affected, was possibly the result of the use of drug in patients. In addition to the three BP, the processes of signal transduction, cell differentiation, nervous system development, and immune response affected more than 5 genes. The down-regulated genes were enriched within synaptic transmission, DNA-dependent regulation of transcription, and vesicle docking during exocytosis, etc (Table 2). It was interesting that both up-regulated and down-regulated genes were enriched within the following biological processes: DNA-dependent regulation of transcription, development, and signal transduction, suggesting that the three BP are significantly affected in the course of PD.

The genes with a same expression trend in cortex and blood, and the genes with 1.4-fold higher expression level are listed in Table 3. The GFAP gene was most significantly up-regulated and its level was 1.7-fold higher in cortex and 1.6-fold higher in blood in PD patients than in normal controls. The GFAP was a marker that can distinguish astrocytes from other glial cells during development, indicating that the astrocytes are affected in the early stage of PD. The

Table 1. Biological Processes of Up-regulated DEG in both Cortex and Blood

Biological Process	Count	*P*-Value	*Q*-Value	Protein
GO:0007275 development	11	1.33E-10	1.64E-10	ANGPT1; CSRP2; FGF2; FZD9; MTL5; CDX4; MAB21L1; SEMA3A; HEYL; TNFRSF12A; APOLD1
GO:0042493 response to drug	4	8.58E-09	8.58E-09	BCAR3; RAD54L; SLC1A3; SEMA3C
GO:0006355 regulation of transcription, DNA-dependent	9	2.88E-08	2.70E-08	AEBP1; SALL1; TAF4; LHX2; BCL3; CDX4; CEBPD; BAZ1A; HEYL
GO:0007165 signal transduction	9	1.05E-06	7.32E-07	BDKRB1; ANGPT1; CD33; FGF2; LALBA; ROS1; BCAR3; IL18R1; RIN3
GO:0030206 chondroitin sulfate biosynthesis	2	2.23E-06	1.43E-06	CHST3; CHST11
GO:0030154 cell differentiation	6	2.48E-06	1.53E-06	ANGPT1; CSRP2; MTL5; SEMA3A; TNFRSF12A; APOLD1
GO:0042742 defense response to bacterium	3	3.74E-06	2.22E-06	LALBA; BCL3; GNLY
GO:0007399 nervous system development	5	4.36E-06	2.49E-06	FGF2; FZD9; LHX2; SEMA3A; HEYL
GO:0042088 T-helper 1 type immune response	2	5.26E-06	2.63E-06	BCL3; EBI3
GO:0006955 immune response	5	6.04E-06	2.93E-06	IL1R2; IGSF6; SEMA3C; CCL27; LILRB1

IL-3 gene level was 1.8-fold higher in cortex and 1.4-fold lower in blood in PD patients than in normal controls. IL-3 is involved in a variety of cell activities such as cell growth, differentiation, apoptosis, immune response, and neurotrophic activity, indicating that the immune system plays an important role in the pathogenesis of PD[9].

Although the reason for PD is unknown, its early diagnosis would greatly help its treatment.

It is important to find the marker genes in blood but not in brain since they can be used in early diagnosis of PD. The gene expression pattern showed a significant difference between cortex and blood samples. However, 181 DEG showed a similar

Table 2. Biological Processes of Down-regulated DEG in both Cortex and Blood

Biological Process	Count	*P*-Value	*Q*-Value	Protein
GO:0007268 synaptic transmission	4	8.46E-07	1.24E-06	GABRD; GRIK1; SNAP25; TAC1
GO:0006355 regulation of transcription, DNA-dependent	7	2.21E-06	2.79E-06	ZNF493; SIX3; ZNF238; TBR1; MKL2; RBPJL; CBX6
GO:0006904 vesicle docking during exocytosis	2	1.27E-05	1.21E-05	STXBP1; VPS33B
GO:0051016 barbed-end actin filament capping	2	1.53E-05	1.38E-05	EPB49; SPTBN2
GO:0007420 brain development	3	1.67E-05	1.38E-05	NNAT; SIX3; TBR1
GO:0045944 positive regulation of transcription from RNA polymerase II promoter	3	3.32E-05	2.44E-05	LIF; SIX3; MKL2
GO:0007275 development	6	3.44E-05	2.44E-05	LIF; ENC1; NNAT; SIX3; MKL2; KAZALD1
GO:0006811 ion transport	4	3.51E-05	2.44E-05	CLCNKB; GABRD; GRIK1; KCNJ3
GO:0007165 signal transduction	7	3.53E-05	2.44E-05	GABRD; BCAM; PPP2R5B; TRHDE; RBPJL; OPN3; BAIAP2L2
GO:0007269 neurotransmitter secretion	2	3.59E-05	2.44E-05	SNAP25; LIN7B

Table 3. DEG with a Same Trend in Both Cortex and Blood

Gene Name	Accession	Cortex			Blood		
		Patient	Control	Ratio	Patient	Control	Ratio
GFAP	NM_002055	1924.9±905.8	1574.8±834.7	1.7	41.2±28.1	34.8±23.3	1.6
SERPINA3	NM_001085	162.6±225.8	100.4±134.6	1.6	50.5±29.9	37.5±23.6	1.7
TNFRSF12A	NM_016639	32.3±23.0	19.7±11.3	1.5	85.2±53.3	81.7±67.7	1.5
GALNT3	NM_004482	14.5±7.0	9.9±3.7	1.5	23.6±19.0	19.7±13.8	1.5
LHX2	NM_004789	746.7±274.1	569.1±195.7	1.5	6.5±5.6	3.8±2.1	1.5
CA12	NM_001218	19.5±20.9	11.5±5.4	1.5	41.7±21.6	26.4±17.1	1.6
SALL1	NM_002968	34.1±11.3	23.7±7.7	1.4	12.9±12.3	8.5±6.7	1.5
GNLY	NM_006433	10.9±7.9	10.9±10.1	1.4	722.5±476.6	654.8±420.0	1.5
IL3	NM_000588	5.0±2.7	55.2±247.6	-1.8	6.0±5.7	9.7±9.0	-1.4
Familial Mediterranean fever locus	AF098968	9.9±4.7	13.5±6.1	-1.6	15.8±13.5	17.0±17.1	-1.6
CDH16	NM_004062	3.6±1.2	5.0±1.6	-1.6	26.1±26.7	29.9±19.7	-1.5
TAC1	NM_003182	54.0±46.0	64.9±34.1	-1.5	8.9±7.6	9.6±8.1	-1.5
BCAM	NM_005581	1127.9±487.8	1428.6±470.1	-1.5	18.0±15.5	21.5±13.8	-2.0
SLC6A11	NM_014229	3.7±1.4	4.3±1.7	-1.5	25.1±17.9	30.0±16.2	-1.4
KRT81	NM_002281	245.9±80.5	316.3±88.4	-1.5	7.2±5.4	13.9±11.3	-1.5

Note. Normalized signals (mean±SD) and average ratio (patient/control) are listed.

tendency in cortex and blood samples from PD patients and can be considered as candidates for the early screening gene markers of PD in blood, or at least would guide the finding of early screening genes. The analysis of expression profiles in this study suggested that DEG with a same trend were enriched in BP related to development and regulation, including DNA-dependent regulation of transcription, development, and signal transduction.

The GFAP expression level was significantly higher in cortex, than in blood, and significantly higher in other neurodegenerative diseases like FFI[5] and genetic Creutzfeldt-Jakob disease[6], suggesting that the damaged astrocytes may be the common features of neurodegenerative diseases. When we compared the DEGs in cortex and in blood in previous studies directly[7-8], we did not find the overlapped DEGs with our results. Those tissue-specific DEGs could not represent the significant expression level of the other tissues. Our study pointed out the genes that have the similar expression trends in both blood and brain tissues, and possibly have less tissue-specific bias for the chosen of potential marker genes.

*This work was supported by the National Natural Science Foundation of China (81101302, 31270185), SKLID Development Grant (2014, SKLID201).

#Correspondence should be addressed to: WANG Zhao Yang, Tel: 86-10-64287525, Fax: 86-10-64287525, E-mail: xlywzy@163.com; and LIU Di, Tel: 86-10-64807385, Fax: 86-10-64807426, E-mail: liud@im.ac.cn

△The first two authors contributed equally to this article.

Biographical notes of the first authors: ZHANG Yi, male, born in 1981, PhD candidate, majoring in bioinformatics; YAO Li, female, born in 1981, physician, majoring in molecular pathology.

Received: May 4, 2014;
Accepted: May 29, 2014

REFERENCES

1. Shulman JM, De Jager PL, and Feany MB. Parkinson's disease: genetics and pathogenesis. Annu Rev Pathol, 2011; 6, 193-222.
2. Lees AJ, Hardy J, and Revesz T. Parkinson's disease. Lancet, 2009; 373, 2055-66.
3. Dong JQ, Zhang ZX, Zhang KL et al. Parkinson's disease and smoking: an integral part of PD's etiological study. Biomed Environ Sci, 2003; 16, 173-9.
4. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry, 2008; 79, 368-76.
5. Tian C, Liu D, Sun QL, et al. Comparative analysis of gene expression profiles between cortex and thalamus in Chinese fatal familial insomnia patients. Mol Neurobiol, 2013; 48, 36-48.
6. Tian C, Liu D, Chen C, et al. Global transcriptional profiling of the postmortem brain of a patient with G114V genetic Creutzfeldt-Jakob disease. Int J Mol Med, 2013; 31, 676-88.
7. Scherzer CR, Eklund AC, Morse LJ, et al. Molecular markers of early Parkinson's disease based on gene expression in blood. Proc Natl Acad Sci USA, 2007; 104, 955-60.
8. Dumitriu A, Latourelle JC, Hadzi TC, et al. Gene expression profiles in Parkinson disease prefrontal cortex implicate FOXO1 and genes under its transcriptional regulation. PLoS Genet, 2012; 8, e1002794.
9. Wang L, Xie Y, Zhu LJ, et al. An association between immunosenescence and CD4(+)CD25(+) regulatory T cells: a systematic review. Biomed Environ Sci, 2010; 23, 327-32.