行政院國家科學委員會專題研究計畫　成果報告

精神分裂症患者血中 IL-6 濃度，DISC1 基因及 IL-6 基因多型性及持續注意力缺損的相關研究

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精神分裂症患者血中 IL-6 濃度, DISC1 基因及 IL-6 基因多型性及持續注意力缺損的相關研究

中英文摘要：

關鍵詞: 精神分裂症的斷裂基因-1，精神分裂症，關聯性研究，持續性注意力，基因表現，基因調控，轉譯因子，白細胞介素六號(IL-6)，單一核酸多型性

精神分裂症是一重大慢性的神經精神疾病，其病因目前仍不清楚，但確定有明顯的遺傳因素。全基因體掃描發現在第 1 對染色體長臂 41-42 區域（1q41-42）與精神分裂症有連鎖證據，過去本研究團隊在台灣的精神分裂病人樣本也發現類似的連鎖證據。精神分裂症的斷裂基因-1 (DISC1 gene)是位於這位址的一個候選基因，與神經元的生長與神經突觸的發展有關，本研究團隊也發現台灣的精神分裂症患者與這個基因有顯著的關聯性，特別是在持續注意力不佳的精神分裂症患者身上，關聯性特別顯著。

有報告發現精神分裂症病人的血清中的 cytokines 較正常對照組為高，有報告的包括 interleukine-1(IL-1), interleukine-2 (IL-2), interleukine-6 (IL-6)等，我們以生物資訊的工具預測發現在 DISC1 gene 的 TATA box promoter region 可以被 interleukine-6 (IL-6) 的 transcription factor 調節表現.，我們假設 IL-6 透過調節 DISC1 基因的作用，與精神分裂症的持續注意力缺損的症狀可能有相關。

本研究計畫內容主要分為三部分，第一部分在於收集 100 位精神分裂症個案及 100 位年齡性別相符的正常對照組，抽血取其 DNA 及血清，並作持續性注意力測驗(continuous performance test, CPT)，第二部分在測量其血清的 cytokines 濃度，及定出 IL-6 基因及 IL-6 受體的 SNP 的定基因型工作。第三部分則於神經母細胞瘤細胞株培養時外加 IL-6，測細胞 DISC1 m-RNA 的表現量的改變。

本研究計畫的主要目的有：(1) 探討精神分裂症患者血清中 cytokines 濃度在精神分裂症者與正常對照組之間是否有差異? (2) 探討精神分裂症患者與 IL-6 基因及 IL-6 受體基因的 SNPs 有無顯著關聯性? 以及 IL-6 基因及 IL-6 受體基因的 SNPs 與 CPT 的表現是否有顯著關聯性? (3) 探討表現 DISC1 蛋白質的神經母細胞瘤細胞株，其調控機制與 IL-6 之間的關係。

本研究的主要結果有：(1) 在 100 位精神分裂症患者及 100 位正常對照個案間的 interleukines，包括 IL-1A, IL-1B, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-alpha, GM-CSF, interferon-alpha, 除了 interferon-alpha 在精神分裂症患者達到邊緣性顯著較低外($p=0.058$)，其餘 cytokines 在兩組間均無顯著差異 (2) 以 100 位精神分裂症患者及 113 位正常對照個案為樣本，發現精神分裂症患者與 IL-6 基因及 IL-6 受體基因的 SNPs 沒有顯著關聯性，且 IL-6 基因及 IL-6 受體基因的 SNPs 與 CPT 的表現也無顯著關聯性 (3) 研究發現神經母細胞瘤細胞株在外加 IL-6 後，會造成 DISC1 long-form isoform m-RNA 的表現量降低。

Keywords: DISC1, Schizophrenia, Association study, continuous performance task (CPT), gene expression, gene regulation, IL-6, Single nucleotide polymorphism (SNP)

Schizophrenia is a major and chronic neuropsychiatric disorder. The etiology is still unknown. The genetic factor plays an important role in the pathogenesis. Previous genome-wide scans studies revealed suggestive linkage evidence of schizophrenia to chromosome 1q41-42. Our research team also replicated this finding in Taiwanese schizophrenic families. Disrupted in schizophrenia-1 (DISC-1) gene is a potential candidate gene on this region and its function is related to the neuron outgrowth and synapse development. We also found significant association between DISC1 and Taiwanese schizophrenic patients, especially in those with sustained attention impairment.

Many reports have shown the serum cytokine levels, including IL-1, IL-6 TNF-alpha, etc., are elevated in schizophrenia than in normal controls. We found a binding site of interleukine-6 (IL-6) transcription factor around the TATA box promoter region of DISC1 gene through transcription factor prediction software. We hypothesized that the IL-6 may be related to the attention deficits of schizophrenia through the regulation of DISC1 expression.

The main work of this project included three parts: (1) we prepared to collect the serum and DNA sample of 100 schizophrenics and 100 age-sex matched normal controls. All the cases and controls will receive Continuous Performance Test (CPT). (2) The laboratory work focus upon measuring cytokine serum concentration and genotyping the SNPs of IL-6 gene and IL-6 receptor gene in this sample. (3) We will measure the change of DISC1 expression through administration of IL-6 in neuroblastoma cells.

The main results include: (1) there are no significant differences in the serum level of cytokines between 100 schizophrenics and 100 normal controls, including IL-1A, IL-1B, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-alpha, GM-CSF. There is marginal significant decrease in schizophrenics compared to normal controls in the serum level of Interferon-gamma ($p=0.058$). (2) there is no significant association evidence between the IL-6 gene and IL-6 receptor gene SNPs and schizophrenia using 100 schizophrenics and 113 normal controls as sample. There is also no significant association between the two genes and CPT performance. (3) We found the m-RNA of long-form isoform of DISC1 significantly decreased after administration of IL-6, but other isoforms seem not significant change after IL-6 treatment.

研究目的

The specific aims of this project are (1) to assess the difference of serum level of IL-6 between schizophrenia and normal controls; (2) to assess the possible association between schizophrenia and IL-6 gene and IL-6 receptor gene; (3) to clarify the possible role of IL-6 in the expression of DISC-1 in neuroblastoma cell lines.

文獻探討

Elevated circulating levels of IL-6 in schizophrenia

Schizophrenia is a heterogeneous mental disorder affected 1% of all populations. The disease mainly affected the brain system, however, immune alterations and genetic predispositions have also been studied intensively due to the interaction of predisposed genetic effects and various environmental influences [1-3]. In the predisposed immunological genetics, proinflammatory (IL-1β, IL-6 and TNFα) and anti-inflammatory (IL-1RA and IL-10) genetic polymorphism have been found positive associations with schizophrenia in the IL-1β–511C/T (2q13), IL-10 promoter polymorphic haplotype (1q32.1), TNFα (6p21.33) and IL-1RA (86bps)n repeats (2q13) in the Finnish, and the Italian ethnic groups [4-7]. In the peripheral levels of immunological factors, such as T-helper cells [7, 8], antibodies to heat shock proteins [9, 10], tumor necrosis factor-a (TNFα) [7, 9, 11-14], and accumulative evidence of interleukines and their receptors [4, 11-23], have been found involving in schizophrenia. Within all these immunological factors, interleukine-6 (IL-6) is the factor most consistently found related to schizophrenia [11, 17, 24-26].

IL-6 is a pleiotropic cytokine released both from peripheral immune cells and from neurons and microglias of the central nervous system (CNS) [27, 28]. In CNS, IL-6 decreased rat embryonic neuronal survival rate [19]. Soluble IL-6 receptor (sIL-6R) levels in the CSF have increased in schizophrenic patients with marked paranoid-hallucinatory syndrome [29]. In peripheral, IL-6 have been found elevated persistently in the plasma of the patients in different ethnic groups [20, 26, 30, 31]. High IL-6 levels have been found relating to the duration and to treatment resistance of schizophrenia [20, 31]. These results suggest that the role of IL-6 elevating in the plasma level of schizophrenia is an unfavorable course of the disease: longer duration of illness, greater treatment resistance, or more marked paranoid-hallucinatory

symptomatology.

The possible role of IL-6 in the pathological process of schizophrenia has been suggested as mediating through the increment of T-helper cells, IL-4 and IL-10 (Fig. 1). The plasma IL-6 is not relating to the birth of different seasons [32], nor has shown differences between schizophrenia and controls at the late pregnancy women plasma levels. Among these searches, none of the study has been done on the role of IL-6 to the attention deficit of the patients.

DISC1 gene associated with schizophrenia may be regulated by IL-6

Many chromosome regions have shown suggestive evidences for linkage with schizophrenia. These suggestive loci include chromosome 1q21-q22, 1q31-q42, 2p22-q21, 4q24-q32, 6p24-p22, 6q16-q23, 8p24-p21, 10p14-p13, 13q14-q32, 15q13-q14, 22q11-q13 [36-44]. One of the promising chromosome regions is located at chromosome 1q42 [45-51]. In this region, a balanced translocation (1; 11)(q42.1; q14.3) has been found associated with schizophrenia in a five generations of a large Scottish pedigree [52]. Family members carried this translocation develop different kinds of mental illness, including schizophrenia. This translocation has disrupted a gene, disrupted-in-schizophrenia 1 (DISC1), which causes the protein malfunction after translation.

Besides the disruption finding, a fine mapping in a Finnish family sample has reported strong linkage evidence (Zmax = 3.21) to the marker D1S2709, where it is located between the exon 4 and exon 5 of DISC1 at chromosome 1q42.1 [53]. Our previous study, in the population of Taiwan, has also suggested a marker of D1S251 close to the promoter region of the DISC1 gene linkage with the disease [49]. In further fine mapping study of this region found significant association evidence between DISC1 and Taiwanese schizophrenia, especially in those with sustained attention impairment. We found a binding site of interleukine-6 (IL-6) transcription factor around the TATA box promoter region of DISC1 gene through transcription factor prediction software. We hypothesized that the IL-6 may be related to the attention deficits of schizophrenia through the regulation of DISC1 expression.

研究方法

Patient criteria, CPT test, and blood sample collection

1. Collection of DNA and serum of schizophrenic patients

We prepare to collect the DNA and plasma of 100 schizophrenic patients from the psychiatric inpatient ward and outpatient clinic of National Taiwan University Hospital and 100 normal controls hospital staff of National Taiwan University

Hospital. All the patients and controls will be screened for the presence of acute infectious disease. After obtaining inform consents, all patients and controls met the screen criteria will be drawn about 10 cc blood from the antecubital vein. In the same time, patients will receive clinical interview with Psychiatric Diagnostic Manual (Hwu et al. 1991) and CPT test to verify the diagnosis of DSM-IV schizophrenia. The normal controls will receive a screening schedule for ruling out major psychiatric illness and substance abuse.

Genotyping methods of SNPs of IL-6 gene and IL-6 receptor gene

1. Serum and DNA purification

After obtaining the whole blood sample, separation of the serum and blood cells will be performed immediately. The serum will be stored in the -20℃ refrigerator as soon as possible for further cytokine analysis. The blood cells will be used for purification of DNA. We used DNA purification kits (Gentra™) for DNA extraction.

2. Genotyping methods of SNPs of IL-6 gene and IL-6 receptor gene

We selected 3 SNPs of IL-6 gene and 3 SNPs of IL-6 receptor gene from Public SNP databank. All SNPs will be genotyped by the method of direct sequencing.

Cytokines assay

The plasma concentrations of GM-CSF, IL-10, IL-12, IL-1a, IL-1B, IL-2, IL-4, IL-6, IL-8, INFg, TNFa were measured by the Beadlyte Human Multi-cytokine detection system (Upstate, U.S.A.). Concentrations of 500 pg/ml of each cytokine standard mixture were four-time serial diluted with assay buffer according to the manufacturer protocol to make a standard curve. Human serum sample of 25 ul was added with 25 ul of serum diluents to incubate with 25 ul of Beadlyte Anti-Human Multi-Cytokine beads solution on a 96-well filter microplate. After an overnight incubation, the beads solution was filtered and washed with assay buffer by vacuum the plate through a MultiScreen vacuum manifold. A 25 ul of Beadlyte anti-human multi-cytokine biotin of secondary antibody solution was incubated with the cytokine-binding beads on the plate for 1.5 hours at dark. At the end of incubation, a 25 ul of Beadlyte streptavidin-phycoerythrin was added into each well, and incubated for 30 min at 25℃ at dark. The reaction was stop by 25 ul of stop solution. The beads on the plate were resuspended in 75 ul of assay buffer and the fluorescent intensity on the beads of each cytokine was measured by Luminex 100 system IS total system (xMAP technology, U.S.A.).

Real time PCR

Total RNA extraction

SV total RNA isolation system (Promega Corporation, Madison, WI, U.S.A.) was used according to the manufacturer's guidelines to extract total RNA from the cultured IMR-32 and EBV-transformed lymphoblasts. The cells after PBS wash were lysed in SV RNA lysis buffer and diluted with SV RNA dilution buffer to make a clear lysate solution. The lysate solution was added with 95% ethanol and transferred into a spin column assembly. The RNA binding on the column membrane was washed with SV RNA wash solution by 12000 rpm centrifugation for 1 min and incubated with DNase for 15 min at 20-25℃ to remove DNA. After DNase incubation, the column membrane was washed with SV DNase stop solution to terminate the reaction and washed with SV RNA wash solution before elution. The total RNA was eluted with 100 ul of nuclease-free water for further experiments. A housekeeping gene, glucuronidase, was used to assess the quality and abundance of RNA for each tissue sample.

RT-PCR

A 13 μl reaction volume of total RNA (3.4 μg of IMR-32 and 10 μg of EBV-transformed lymphoblasts), and 1μl of oligo (dT)15 (0.5 μg/μl) was mixed and heated to 70℃ for 5 min to melt secondary structure within the template. The mixture was quickly chilled on ice, it then added with 5 μl of M-MLV 5x first strain buffer (Promega Corporation, Madison, WI, U.S.A.), 5 μl of 10 mM dNTP mix (10mM each dATP, dGTP, dCTP, and dTTP), 1 μl of M-MLV RT (200 units) and 0.625 μl of RNase inhibitor (25 units). After incubated at 42℃ for 1 hour, the mixture was ready for further polymerase chain reaction (PCR).

For each 10 μl reaction, 1 μl of 10x ProTaq DNA polymerase (Protech Technology, Taiwan) reaction buffer, 0.2 μl of each forward and reverse primer (10 μM each), 0.4 μl of 10 mM dNTP mix, 0.8 μl of ProTaq DNA polymerase, aliquot of water, and cDNA sample (143 ng～572 ng of IMR-32 and 429 ng～858 ng of EBV-transformed lymphoblast) were used. The PCR cylcling parameters were 95°C for 5 min, followed by 40 cycles of 95°C denaturation for 45 sec, 64-69 ℃ annealing for 30 sec, 72℃ of synthesis for 45 sec, and a final extension step at 72℃ for 5 min. The PCR product was examined by 1.5% agarose gel electrophoresis to account for succeed of the PCR reaction.

Real time PCR for the expression of DISC1 isoforms

Probes and primers of four DISC1 isoforms (L, Lv, Es and S isoforms) and a total isoform were designed by Assay-by-Design (Applied Biosystems, Branchburg,

NJ). Real-time RT-PCR amplification was carried out using TaqMan One-Step RT-PCR Master Mix Reagent (Applied Biosystems, Branchburg, NJ) on an ABI PRISM 7900HT Sequence Detection System in triplicates, according to the manufacturer's instructions. Gene expression was quantified relative to the expression of a house-keeping gene; TATA-box binding protein (TBP) using Sequence Detector Software and the relative standard curve method.

The reaction mixture for real-time PCR was prepared with the protocol provided by the manufacturer. For each 20 μl reaction, 10 μl of 2x Master Mix, 6.5 μl of ddH2O, 1 μl of ABD prbe, 0.5 μl of 40x multiscribe and Rnase inhibitor and 2μl RNA sample (20 ng total RNA per μl) were used. The cycling parameters were 50°C for 2 min and 48°C for 30 min to reverse transcribe RNA, followed by 95°C for 10 min to activate the Taq, and 40 cycles of 95°C denaturation for 15 sec and 60°C reannealing/ extension for 1 min.

Statistics Analysis

The comparison of cytokine level between schizophrenia and normal control was performed with two sample t-test. The comparison of allele and genotype frequencies of SNPs for IL-6 gene and IL-6 receptor gene were calculated with chi-square and Fisher exact test. The intermarker linkage dysequilibrium assessed by D' was calculated by Haploview. The comparison of quantitative phenotype of CPT between different genotype and haplotype was calculated by Kruskal-Wallis test. The difference before and after administration of IL-6 to neuroblastoma cell lines was compared using t-test.

Result

We selected 3 SNPs of IL-6 gene [rs1800797 (IL6-1), rs3087233, and rs3087236] and 3 SNPs of IL-6 receptor gene [rs4845617(IL6R-1), rs4553185(IL6R-2), rs4379670(IL6R-3)) from public SNP databank. We found the SNPs rs3087233 and rs3087236 were not valid in our sample. However, we found other two SNPs, which were 38bp (IL6-2) and 63bp (IL6-3) distal from the SNP rs3087236.

Table 1 showed descriptive statistics of the 6 SNPs which we genotyped in 100 schizophrenics and 113 normal controls. Table 2 showed the results of Hardy-Weinberg test and allele-wise and genotype-wise comparison of the six SNPs. The SNP ILR6-2 was incompatible with the Hardy-Weinberg equilibrium in controls. The comparisons between the frequencies of allele and genotypes between case and control were all non-significant.

Table 3 showed the quantitative phenotype comparison, including CPT performance index d', and serum level of IL-6 and interferon-gamma between different genotypes of the six SNPs. All the comparisons are non-significant.

Fig 1 showed the haplotype blocks assessed by HAPLOVIEW. The three SNPs of IL-6 with high intermarker D' formed a haplotype block. The two SNPs (IL6R-2, IL6R-3) with high intermarker D' formed a haplotype block. Table 4 showed the haplotype analysis of the two genes. There was no significant association between the haplotypes and schizophrenia.

Table 5 showed the comparison of serum level of cytokines between 100 schizophrenics and 100 normal controls. There are no significant differences in the serum level of cytokines between schizophrenics and normal controls, including IL-1A, IL-1B, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-alpha, GM-CSF. There is marginal significant decrease in schizophrenics compared to normal controls in the serum level of Interferon-gamma (p=0.058).

Table 6 showed the change of level of DISC1 isoforms m-RNA after administration of different concentration of IL-6 (5 ng/ml, 20 ng/ml) in the neuroblastoma cell line IMR-32. We found the m-RNA of long-form (L) isoform of DISC1 significantly decreased after administration of IL-6, but other isoforms seems not significant change after IL-6 treatment.

Table 1. Descriptive statistics of the 6 SNPs genotyped in 100 schizophrenics and 113 normal controls

SNP	Control				Case				Combined			
	11	*12*	*22*	MAF[a]	*11*	*12*	*22*	MAF	*11*	*12*	*22*	MN[b]
IL6R1	0.16	0.59	0.25	(A)0.46	0.14	0.62	0.24	0.45	0.15	0.6	0.25	2
IL6R2	0.21	0.62	0.17	(T)0.48	0.19	0.64	0.17	0.49	0.20	0.63	0.17	15
IL6R3	0.67	0.30	0.028	(T)0.18	0.63	0.31	0.068	0.22	0.65	0.3	0.046	16
IL6-1	0.61	0.35	0.045	(G)0.22	0.58	0.35	0.07	0.25	0.59	0.35	0.057	1
IL6-2	0.47	0.42	0.11	(G)0.32	0.43	0.43	0.14	0.36	0.45	0.43	0.12	4
IL6-3	0	0.02	0.98	(G)0.01	0	0.01	0.99	0.005	0	0.016	0.98	19

[a]: Minor allele frequency (MAF)

[b]: Missing number (MN)

Table 2. Tests for Hardy-Weinberg equilibrium and genotype-wise and allele-wise comparison

SNP	HWE in control			HWE in case			Association test (p-value)		
	n [a]	Asy. [b]	Exact[c]	*n* [a]	Asy.[b]	Exact[c]	Geno	Allele	Trend
IL6R-1	112	0.05	0.05	99	0.01	0.03	0.90	0.90	0.89
IL6R-2	112	0.013	0.02	86	0.01	0.02	0.89	0.74	0.71
IL6R-3	109	0.75	1.00	88	0.30	0.35	0.38	0.29	0.30
IL6-1	112	0.84	1.00	100	0.59	0.59	0.72	0.52	0.53
IL6-2	109	0.74	0.82	100	0.54	0.52	0.76	0.46	0.47
IL6-3	095	0.92	1.00	99	0.96	1.00	0.54	0.54	0.54

[a]: Number of individual

[b]: P-value of Chi-Square

[c]: P-value of exact test

Table 3. Test for quantitative phenotype by Kruskal-Wallis Test

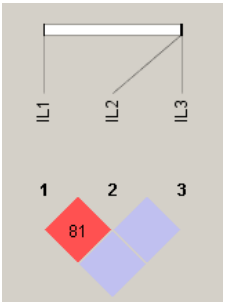
Phenotype	**SNPs (p-value)**							
	Case (LP, N=100)							
	ILR6-1	ILR6-2	ILR6-3	IL6-1	IL6-2	IL6-3	IL6-H*	IL6R-H*
#ZDPRIME	0.36	0.49	0.26	0.54	0.77	0.58	0.56	0.72
#ZMDPRIME	0.49	0.94	0.61	0.38	0.15	0.14	0.13	0.48
$IL6	0.62	0.66	0.65	0.73	0.33	0.94	0.81	0.72
$INF-gamma	0.32	0.12	0.31	0.28	0.18	0.51	0.38	0.46
	Control (NC, N=113)							
	ILR6-1	ILR6-2	ILR6-3	IL6-1	IL6-2	IL6-3	IL6-H*	IL6R-H*
ZDPRIME	0.28	0.24	0.86	0.36	0.57	0.58	0.73	0.85
ZMDPRIME	0.40	0..08	0.26	0.22	0.35	0.35	0.40	0.29
IL6	0.77	0.48	0.25	0.6	0.89	0.34	0.29	0.24
INF-gamma	0.53	0.82	0.52	0.10	0.05	0.78	0.18	0.63

#ZDPRIME and ZMDPRIME means the standized z score of d' of undegraded and degraded CPT, respectively. *ILH: IL6 haplotype; ILRH: IL6 receptor haplotype. $IL6, INF-gamma: the serum level of IL-6 and interferon-gamma

Phenotype data 存在 Case or Control 中,故以 Kruskal-Wallis Test 分別檢定在 case and control 中 genotype 爲 11,12,22 三組人的 phenotype 有否差異。

Fig.1 Haplotype blocks of IL-6 gene and IL-6 receptor gene measured by HAPLOVIEW

IL-6 gene



IL-6 receptor gene

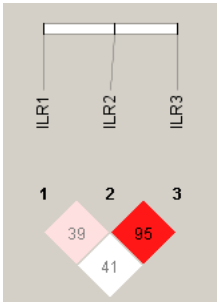


Table 4. Haplotype analysis of the association for IL-6 gene and IL-6 receptor gene

1. IL6

Haplotype Frequencies

Number	Haplotype	Freq	Standard Error	95% Confidence Limits	
1	C-A-G	0.00000	0.00007	0.00000	0.00014
2	C-A-T	0.63398	0.02342	0.58807	0.67989
3	C-G-G	0.00757	0.00421	0.00000	0.01583
4	C-G-T	0.12732	0.01621	0.09555	0.15908
5	G-A-T	0.02766	0.00797	0.01203	0.04329
6	G-G-T	0.20347	0.01957	0.16510	0.24183

Test for Marker-Trait Association

Trait Number	Trait Value	Num Obs	DF	LogLike	Chi-Square	Pr > ChiSq
1	0	100	5	-160.93303		
2	1	113	5	-182.12162		
	Combined	213	5	-344.44902	5.5774	**0.3495**

2. IL6 receptor

Haplotype Frequencies

Number	Haplotype	Freq	Standard Error	95% Confidence Limits	
1	C-A	0.32857	0.02295	0.28360	0.37354
2	C-T	0.18990	0.01916	0.15235	0.22746
3	T-A	0.47660	0.02440	0.42878	0.52442
4	T-T	0.00493	0.00342	0.00000	0.01163

Test for Marker-Trait Association

Trait Number	Trait Value	Num Obs	DF	LogLike	Chi-Square	Pr > ChiSq
1	0	100	3	-147.77540		
2	1	113	3	-173.09925		
	Combined	213	3	-322.17359	5.1958	**0.1580**

Table 5. Comparison of cytokine level between schizophrenia (n=100) and normal control (n=100)

Cytokine	Case (mean±SD)	Control (mean±SD)	t-value	Degree of freedom
IL-1A	18.50±76.23	9.18±34.91	1.11	138.77
IL-1B	12.35±49.46	8.90±14.41	0.67	198
IL-2	6.96±42.28	4.53±22.64	0.51	198
IL-4	1.49±4.98	1.48±2.61	0.015	198
IL-6	8.40±13.69	20.93±83.46	-1.48	104.35
IL-8	2.86±21.41	7.33±21.33	-1.48	198
IL-10	6.29±7.55	8.85±20.75	-1.16	124.76
IL-12	17.02±44.01	27.24±104.48	-0.90	198
INF-gamma	14.42±44.23	33.46±89.05	-1.91#	145.05
TNF-alpha	2.12±7.64	2.23±4.04	-0.12	198
GM-CSF	18.34±62.66	28.21±89.31	-0.9	198

p=0.058

Table 6. IL-6 treatment for three days on DISC1 isoform expressions (△CT) in human neuroblastoma cell line IMR-32.

△CT	DISC1-L	DISC1-Lv	DISC1-S	DISC1-ES	DISC1-Total
Control	0.009±0.0017	0.044±0.0006	0.035±0.0001	0.022±0.006	0.0095±0.002
IL-6 (5 ng/ml)	0.005±0.0011*	0.041±0.004	0.034±0.0042	0.017±0.002	0.0087±0.001
IL-6 (20 ng/ml)	0.006±0.0007*	0.042±0.003	0.031±0.001	0.015±0.003	0.0123±0.0004

Data were presented in mean ± standard deviation.

* represents the p-value less than 0.05 between control and the dose of IL-6

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