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Case Report

Differential protein expression in an acute disseminated myelitis patient after treatment with umbilical cord mesenchymal stem cells

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Abstract: In this study, we report a 63-year-old woman with acute disseminated myelitis who was treated with umbilical cord mesenchymal stem cells (UC-MSC) transplantation. This acute disseminated myelitis patient received UC-MSC transplantation 4 times ($1*10^9$) totally. We extracted the patient's cerebrospinal fluid (CSF) and identified the differentially expressed genes and proteins. We found 116 differentially expressed proteins between samples S1 (Pre-treatment) and S2 (Post-treatment) which commonly expressed in N1 and N2 (volunteers). The differentially expressed proteins include Vascular cell adhesion protein 1 (S2:S1=1.73), intercellular adhesion molecule 5 (S2:S1=1.40), fibroblast growth factor receptor 1 (S2:S1=1.06), NT-3 growth factor receptor (S2:S1=3.79), hepatocyte growth factor activator (S2:S1=2.02), glia-derived nexin, intercellular adhesion molecule (S2:S1=2.14), Intercellular adhesion molecule 2 (S2:S1=4.37), immune globulin, neurexin-3 (S2:S1=1.16), chitinase-3-like protein (S2:S1=1.16), spondin-1 (S2:S1=1.52), calsyntenin-1 (S2:S1=1.35), leucine-rich glioma inactivated protein-1 (S2:S1=1.26), neuronal pentraxin (S2:S1=1.03), neuroligin-1 (S2:S1=0.44), and cadherin-15 (S2:S1=1.36), etc, which could be useful for angiogenesis and the recovery of the nervous system. It may be the novel therapeutic targets.

Keywords: Umbilical cord mesenchymal stem cell (UC-MSC), transplantation, cerebrospinal fluid (CSF), acute disseminated myelitis, differentially expressed proteins, angiogenesis, neural regeneration

Introduction

Human umbilical cord contains abundant mesenchymal stem cells that are more primitive compared to bone marrow MSCs. Human umbilical cord-derived mesenchymal stem cells (UC-MSCs) are a class of cells with significant self-renewal and multi-lineage differentiation properties. UC-MSCs have strong biological activity and have the ability to differentiate. Repeatedly passaged and amplified UC-MSCs still maintain a strong function, which provide a sufficient source of MSCs for experimental and clinical use. Notably, chemical and neurotrophic factors can induce UC-MSCs to differentiate into neural stem cells. UC-MSCs can also differentiate into oligodendrocyte precursor cells, secrete a variety of nerve growth factors (e.g., VEGF, GDNF, and BDNF), and promote axonal growth. UC-MSC-differentiated cells not only exhibit the morphology and phenotype of oligodendrocyte precursor cells but also perform their corresponding function. Here, we report a 63-year-old woman with acute disseminated myelitis who was treated with UC-MSC transplantations. The patient received UC-MSC transplantation 4 times ($1*10^9$) and recovered very well. We evaluated the protein changes in the patient's cerebrospinal fluid (CSF) and we identified the differentially expressed proteins, which may reveal new therapeutic targets for acute disseminated myelitis.

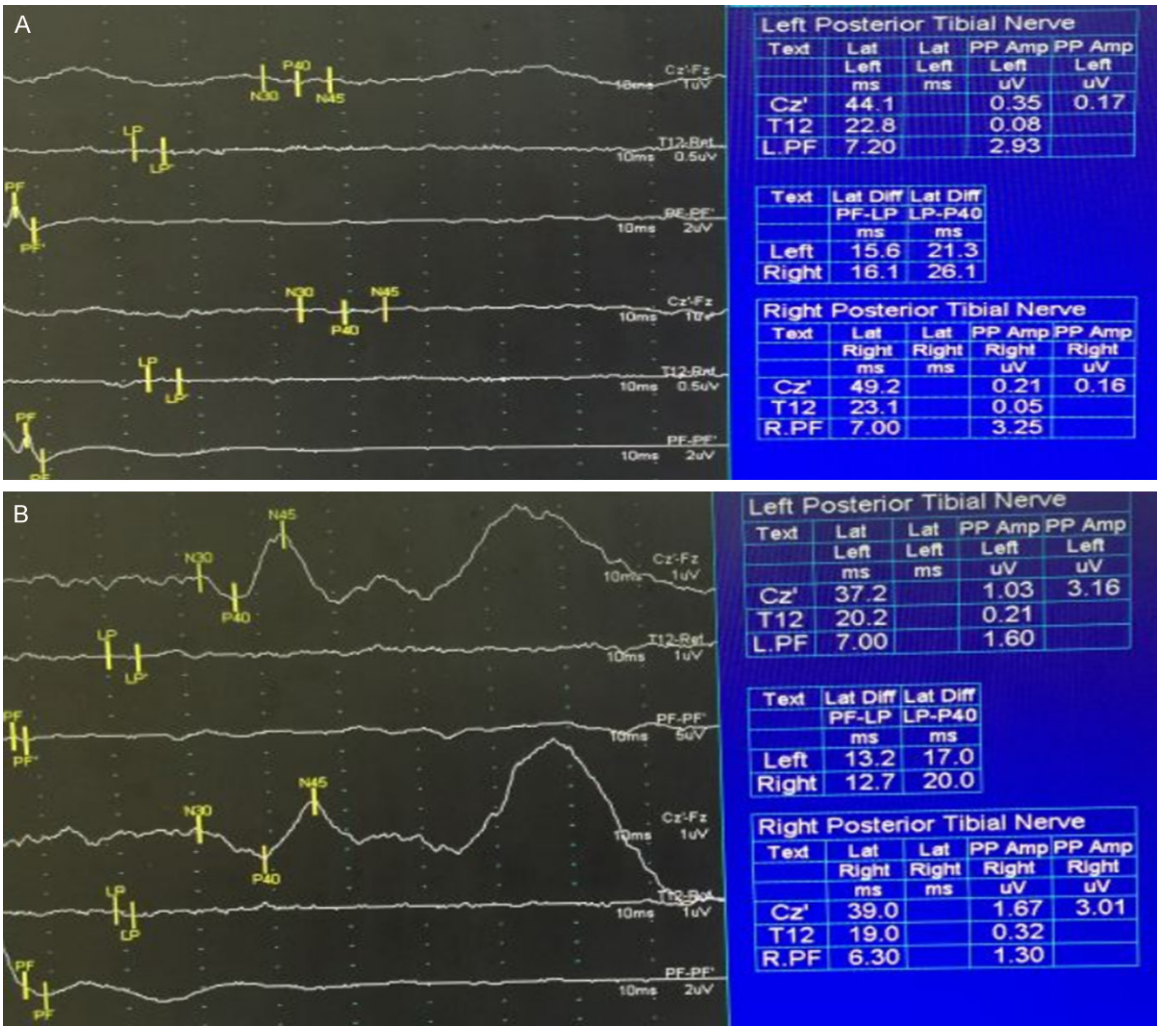

Figure 1. Electromyography-evoked potentials in a 63-year-old female patient with transverse myelitis. A. Electromyography-evoked potentials in the patient revealed: the P40 wave in the latency period was 44.1 ms and 49. 2 ms; the amplitude of the P40 and N45 waves was 0.17 mv and 0.16 mv, respectively. B. 1 month after the second transplantation the electromyography-evoked potentials of the patient are close to normal.

Materials and methods

Preparation of human umbilical cord mesenchymal stem cells

Human umbilical cord was obtained from a woman who gave birth in the obstetrics department of the Affiliated Hospital of the Logistics University of the Chinese People's Armed Police Forces. After the healthy fresh umbilical cord was rinsed with PBS and devascularized and the Wharton's jelly was removed, the entire tissue was separated into 1-mm segments. BioWhittaker Ultra CULTURE™ cell culture medium (general purpose serum-free medium without L-glutamine, Catalog No. 12-725F, Lot No. 0000479259; Lonza, Walkersville, MD21793, USA) was used for mesenchymal stem cell culture and separation in a 5% CO_2 incubator at 37°C. Fourth-generation human umbilical cord mesenchymal stem cells (UC-MSCs) with a cell viability ≥95% and no pathogenic bacteria were used for our experiment.

Ethical considerations

Written informed consent was obtained for the use of the umbilical cord. Our study was approved by the Institutional Review Board of the Affiliated Hospital of the Logistics University of

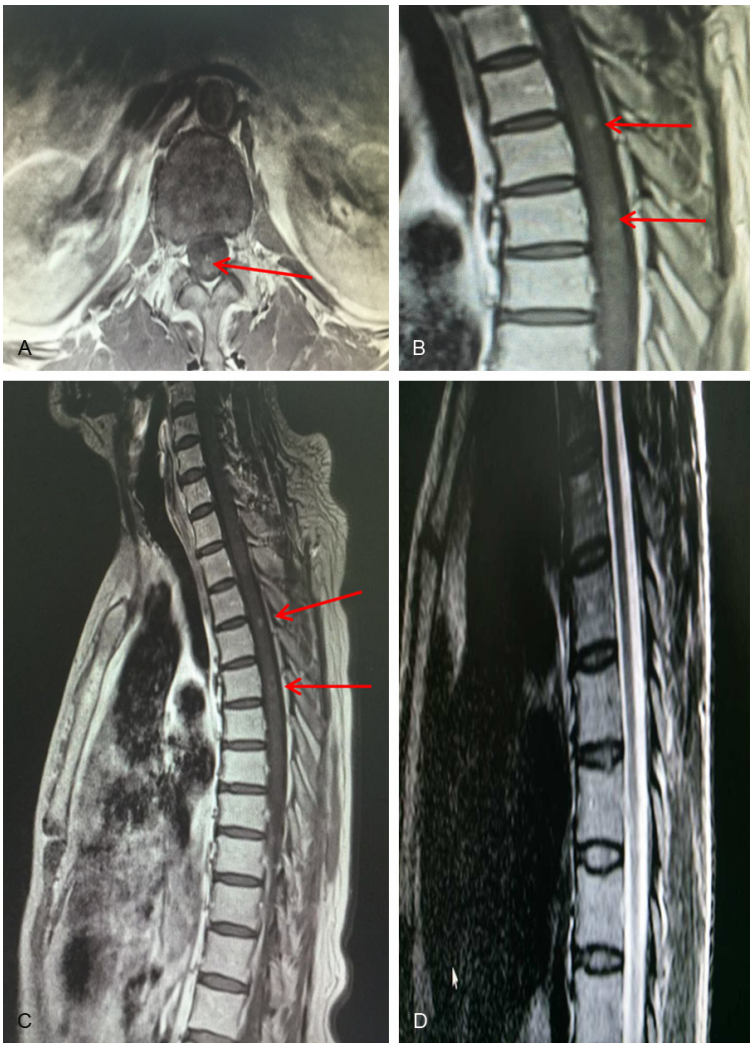

Figure 2. Imaging studies in a 63-year-old female patient with transverse myelitis. A. Horizontal T2-weighted thoracic vertebra enhanced Magnetic Resonance Imaging scans showing cord edema. B, C. Sagittal T2-weighted vertebral column enhanced Magnetic Resonance Imaging scan showing a high-density plot area scattered in the thoracic and lumbar spinal cord with cord edema. D. Horizontal T2-weighted thoracic vertebra enhanced Magnetic Resonance Imaging scans showing cord edema and hyperemia which vanished and showed no significant anomalies 2 months after transplantation.

the Chinese People's Armed Police Forces in 2014. Our animal protocol was approved by the Institutional Animal Care and Use Committee at the Affiliated Hospital of the Logistics University of the Chinese People's Armed Police forces.

UC-MSCs and injection

The human umbilical cord was obtained from a woman who gave birth at the obstetrics department of the Affiliated Hospital of the Logistics University of the Chinese People's Armed Police Forces. The patient received 4 successive intrathecal injections of UC-MSCs that were infused via an intravenous injection (twice, $2*10^8$/time) and an intrathecal injection (twice, $3*10^8$/time) on a bi-monthly basis. Informed consent was obtained from the patient. The Institutional Review Board of the Pingjin Hospital and the academic board of Tianjin University approved the study.

Statistical analysis

Data are expressed as mean ± SD. SPSS 17.0 software was used for statistical analysis. One way ANOVA was performed for the comparison, followed by the Dunnett's test. $P<0.05$ was considered statistically significant.

Results

Clinical findings before and after treatment

In 2016, a 63-year-old female patient developed an acute onset of flu-like symptoms, including fever and weakness in the limbs. Four days later, the patient developed a stationary course of weakness affecting both the upper and lower limbs. She sought immediate medical advice. A clinical examination revealed a high grade fever (38.7°C). The electromyography-evoked potentials of the patient were abnormal, where P40 in the latency period was 44.1 ms and 49.2 ms; the amplitude of P40-N45 was 0.17 mv and 0.16 mv (**Figure 1A**). The patient was conscious, oriented and had no history of convulsions. Her lower limbs had a motor power grade of 1, while the upper limbs showed a greater distal weakness than a proximal weakness (i.e., a distal grade of 2 and a proximal grade of 3). The patient also had difficulty in breathing. Laboratory tests revealed an elevated erythrocyte sedimentation rate (1st hour, 35.6 mm/hour; 2nd hour,

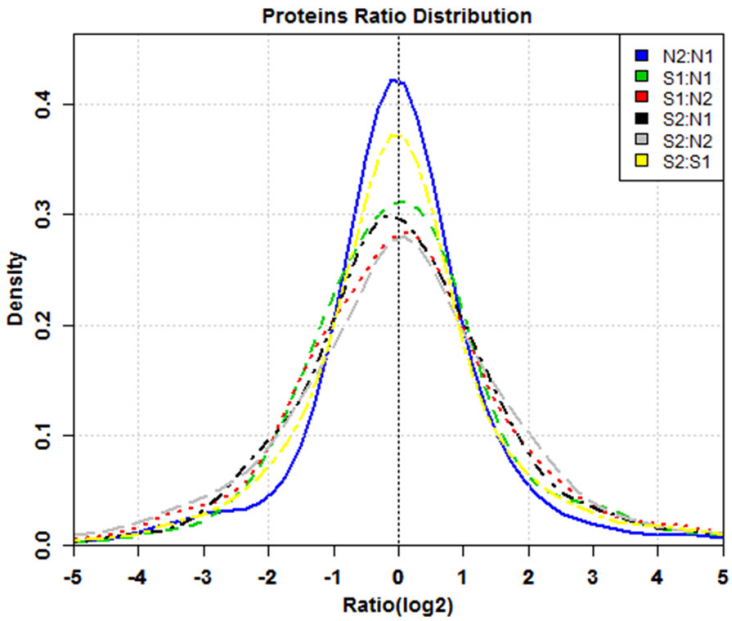

Figure 3. The distribution of volcano figure for the samples ratio. Multiples of the difference were transformed by log base 2. Vertical axis: the *p*-value takes the value of negative log 10.

77.9 mm/hour). Her total leucocyte count was 10,900/μL with a neutrophil count of 13.1%. Her CSF was clear and colorless, with a glucose concentration of 4 mmol/L, a protein concentration of 335 mg/L, and a cell density of 10^7/L. An enhanced Magnetic Resonance Imaging (MRI) showed a scattered high-density shadow in the thoracic and lumbar spinal cord with cord edema (**Figure 2A-C**). The patient received UC-MSCs (2 intravenous injections, $2*10^8$/time and 2 intrathecal injections, $3*10^8$/time) and then prednisolone (oral, 60 mg/d) for 1 month. After the treatment, there was obvious improvement in breathing and the strength in limbs. Electromyography and MRI revealed significant changes, in addition, the changes of proteins in CSF had significant differences. Two months after UC-MSC transplantation, the patient felt well in a good condition with no apparent discomfort. The MRI and electromyographyevoked potentials (**Figures 1B**, **2D**) showed no significant anomalies.

CSF protein changes

We used Sequential Windowed Acquisition of all Theoretical fragment ions (SWATH) [1-8] to detect differential expression proteins in CSF in volunteers and patients. Before transplantation, MSCs need to be dissolved in 1-3 ml normal saline to ensure the balance of intracranial pressure in the progress of transplantation, we extracted the same volume of CSF in order to maintain the balance of intracranial pressure. When the MSCs were transplanted into the cavum subarachnoidale. The samples of CSF were collected and tested. A total of 813 proteins were identified and quantified from the 2 volunteers (N1 and N2) and patient samples (pre- and post-treatment: S1 and S2). Of the 813 proteins, the comparison between S1 and N1, it revealed 316 proteins was changed, of which 146 proteins increased significantly and 170 proteins decreased significantly ($P<0.05$); the comparison between S1 and N2, it revealed 348 proteins was changed, of which 179 proteins increased significantly and 169 proteins decreased significantly ($P<0.05$); the comparison between S2 and N1, it revealed 380 proteins was changed, of which 186 proteins increased significantly and 194 proteins decreased significantly ($P<0.05$); the comparison between S2 and N2, it revealed 410 proteins was changed, of which 211 proteins increased significantly and 199 proteins decreased significantly ($P<0.05$); finally, the comparison between S2 and S1, it revealed that 292 proteins were significantly changed, of which 151 proteins increased and 141 proteins decreased ($P<0.05$).

The difference of protein abundance was more than 1.5 times and a q-value ≤0.05 indicated a significant difference between two samples. The differences were transformed by log base 2, and the expression is more than 0 indicates increased expression, while expression less than 0 indicates decreased expression (**Figure 3**). The clusters of differentially expressed proteins shown in **Figure 4**, it revealed the distribution of differentially expressed proteins, the trend changes, and the putative function of the differentially expressed proteins in all samples, which may be the target genes or proteins in which we are interested.

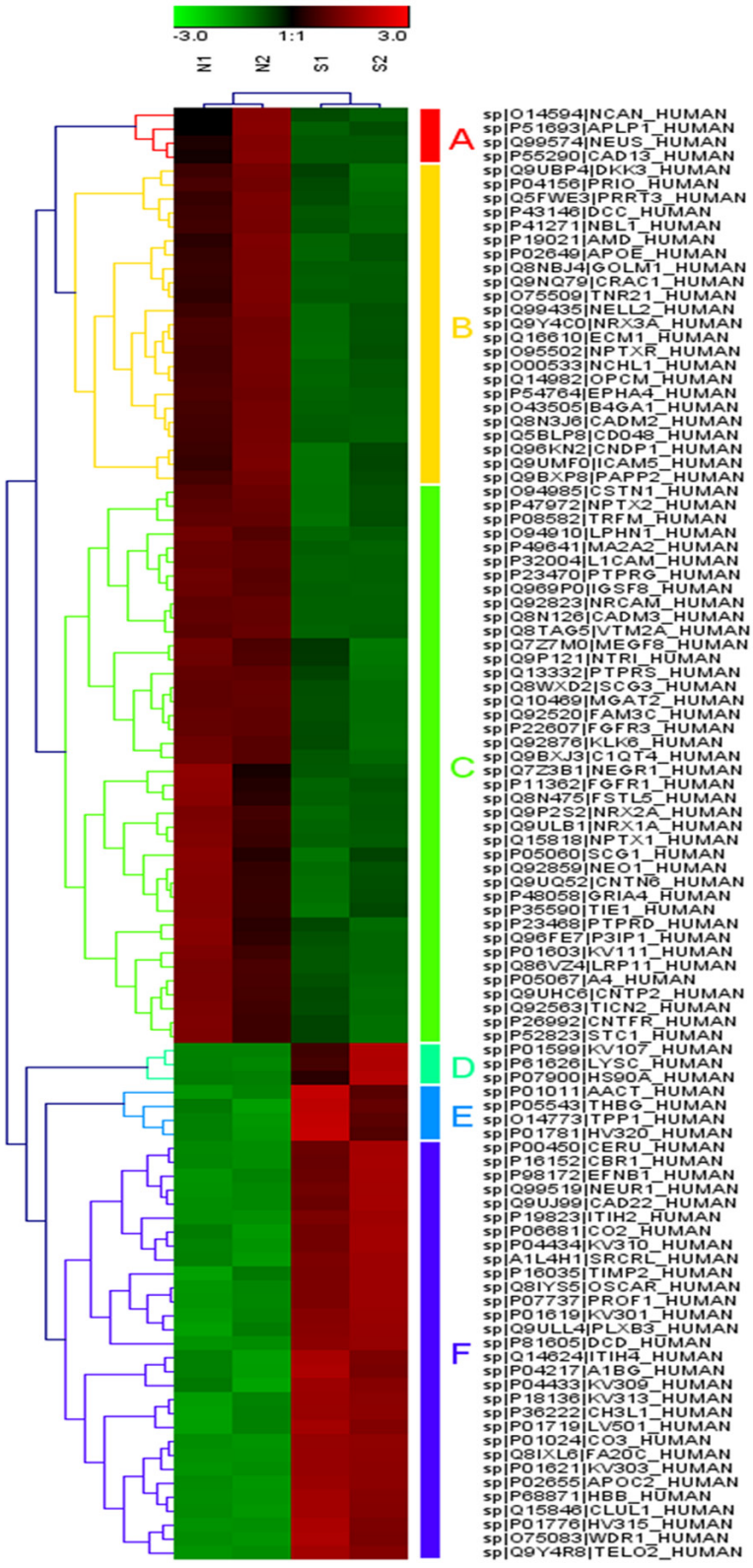

Figure 4. Heat maps of the differentially expressed proteins clustered into groups. The heat map shows the distribution of the differentially expressed proteins across all of the sample, and change trend, and the function of the differentially expressed proteins.

CSF protein changes between samples S2 and S1

A total of 116 differentially expressed proteins were detected between samples S2 and S1, which were commonly expressed in N1 and N2, indicating the differentially expressed proteins after treatment of US-MSC. These proteins may be the targets of treating for acute disseminated myelitis or other nervous system diseases.

Discussion

MSCs are multipotent non-hematopoietic progenitors that are a promising treatment for tissue regeneration [9]. Human umbilical cord-derived mesenchymal stem cells (UC-MSCs) are a class of cells with significant self-renewal and multi lineage differentiation properties. MSCs are abundant in bone marrow, umbilical cord blood, umbilical cord, connective tissue and interstitial tissue [10-13]. Human umbilical cord contains abundant mesenchymal stem cells that are more primitive compared to bone marrow MSCs. UC-MSCs show strong biological activity and differentiation ability. That is, repeatedly passaged and amplified UC-MSCs still maintain a strong function, which provides a sufficient source of MSCs for experimental and clinical use. Several reports indicate that chemical and neurotrophic factors can induce UC-MSCs to differentiate into neural stem cells and oligodendrocyte precursor cells, secrete a variety of nerve growth factors (e.g., VEGF, GDNF and BDNF) and promote axonal growth. These UC-MSC-differentiated

cells not only have the morphology and phenotype of oligodendrocyte precursor cell but also exhibit the corresponding function [14, 15]. UC-MSCs can be also induced to differentiate into mesodermal-originated bone cells, cartilage cells, fat cells, liver cells, myocardial cells and nerve cells. The immune inhibition ability low and immunogenicity of UC-MSCs allows for their use in the treatment of allograft and in xenografts [16, 17].

Mohyeddin Bonab research group [18] conducted a pilot study in ten patients with progressive multiple sclerosis (MS) who did not respond to disease-modifying agents such as mitoxantrone. The patients were injected intrathecally with MSCs. Subsequently, the patients were followed-up with a monthly neurological assessment and MRI scan at the end of the first year. During the 13- to 26-month follow-up period (mean: 19 months), the Expanded Disability Status Scale score of five patients increased from 0.5 to 2.5. A large variety of putative stem cell therapies for MS have been proposed and administered in single cases or in a small series of subjects. For example, the injection of autologous MSCs in animal models of MS was therapeutic [10, 19]. Researchers previously believed that MSCs could differentiate into, and thus replace abnormal neurons or myelinating oligodendrocytes, but the mechanism of action was ultimately found to be immunomodulatory [11, 19-21].

Our patient received UC-MSC transplantation recovered very well and the immune-modulatory properties of these cells, the feasibility of the application were confirmed again [9, 16, 17]. Following UC-MSC treatment, our patient showed obvious improvements in breathing and limb strength with simultaneously significant changes in electromyogram recordings and MRI. In the patient's CSF, we identified the differentially expressed proteins that may be the novel therapeutic targets.

In summary, UC-MSCs have immunomodulatory properties that allow for the using in allograft transplantation. Moreover, UC-MSCs have immunosuppressive properties that make them useful for cell therapy. In our study, we identified the differentially expressed proteins in the CSF of our patient that may be the novel therapeutic targets. We found 116 differentially expressed proteins between S1 and S2 that were commonly expressed in N1 and N2. The differentially expressed proteins include immune globulin, protocadherin gamma-C5, FAM 20C, neurexin-3, chitinase-3-likeprotein, spondin, calsyntenin-1, leucine-rich glioma inactivated protein-1, neuronal pentraxin, neuroligin-1, and cadherin, among others etc, which are useful for the recovery of the patient.

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Disclosure of conflict of interest

None.

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