Changes in urinary proteins of unaffected individuals with a family history of frontotemporal dementia

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Abstract: Frontotemporal dementia (FTD) is a very common type of neurodegenerative dementia and is the main cause of dementia in people under 65 years old. It has strong heritability. To explore whether there are urinary proteins and biological pathways that are different between those who have not been identified with FTD under existing diagnostic methods and healthy people, this study conducts urinary proteomics analysis on people without disease with a family history of FTD and healthy people. The results show that there are 387 significantly different proteins ($P<0.01$; $FC\geq2.0$ or ≤0.5) between the two groups. Among them, many proteins have been reported to play a role in FTD or the nervous system. In particular, Progranulin (PGRN) is considered an effective biomarker for FTD. Among the 139 biological pathways enriched by differential proteins ($P<0.01$), there are many pathways directly related to neurons and the nervous system, including glial cells and microglia. The differences in urinary proteomes between people without disease with a family history of FTD and healthy people make urinary proteomics have the potential to provide clues in exploring potential FTD patient populations and exploring the mechanisms related to the early occurrence and development of FTD, opening up a new perspective for FTD prevention and early diagnosis.

Key words: frontotemporal dementia; family history; urinary proteome

1 Introduction

1.1 Frontotemporal Dementia and Its Research Status

Frontotemporal Dementia (FTD) is a progressive neurodegenerative disease that mainly affects the prefrontal and temporal lobe regions of the brain. These regions are responsible for controlling behavior, language, emotion, and decision-making ability. Therefore, patients with FTD will experience cognitive and behavioral function impairments, language disorders, behavioral and emotional changes, spatial orientation ability decline, and judgment and decision-making ability decline. As time passes, it progresses to more comprehensive dementia and can be accompanied by other movement disorders such as amyotrophic lateral sclerosis[1]. FTD is a very common type of neurodegenerative dementia, which is more common in middle-aged and elderly people aged 45 - 64 years old and is the main cause of dementia in people under 65 years old. Therefore, it is considered an early-onset dementia[2][3]. The disease course of FTD is 2 - 20 years, with an average of about 8 years, and the prognosis is poor. Most patients die from

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complications such as pulmonary infection, urinary tract infection, and pressure sores[4].

The etiology of FTD is not completely clear, but it may be related to genetic and gene mutation factors. Studies have shown that FTD exhibits strong heritability, and 30 - 50% of patients have a positive family history[5][6]. Researchers have identified mutations in the MAPT and progranulin precursor (GRN) genes in approximately 50% of family cases and rare defects in the VCP, CHMP2B, TARDP, and FUS genes in a few families[7][8].

Currently, there is no specific cure for FTD, and the treatment mainly focuses on symptomatic treatment and rehabilitation. The quality of life of patients is improved and the progression of the disease is delayed through comprehensive treatment and nursing measures, including drug treatment (such as acetylcholinesterase inhibitors, anti-anxiety and antidepressant drugs), rehabilitation treatment (such as cognitive training and functional training), behavior therapy, nutritional therapy, family education, psychological counseling, and social participation. Early diagnosis and early intervention can significantly improve the quality of life and prognosis of patients with FTD[9][10][11] .

he clinical diagnosis of FTD is mainly based on neuropsychological assessment, neuroimaging examination, and cerebrospinal fluid and plasma biomarker detection[7][12]. Impaired executive function is a characteristic of FTD. However, in the early stage of the disease, executive dysfunction may not exist or may be masked by obvious behavioral changes. Moreover, due to the similarity of some symptoms in the early stage of FTD and Alzheimer's disease, such as episodic memory impairment, it is difficult to distinguish FTD from AD using standard neuropsychological tests in the early stage. Although existing neuroimaging examination methods can help distinguish FTD from AD, only when the disease progresses to a certain extent and the structural and functional changes of the brain are more obvious can neuroimaging detect abnormalities and cannot play an identification role in the very early stage of the disease. In addition, due to the great differences in pathological heterogeneity and the severity of neurodegeneration, the ability of cerebrospinal fluid biomarkers to reliably identify FTD is limited. Moreover, due to the regulation of the body's homeostatic mechanism, cerebrospinal fluid and blood cannot detect the onset of the disease relatively early[7][13]. Therefore, more sensitive detection methods are needed to achieve earlier diagnosis of FTD.

1.2 Urinary Biomarkers

The urinary proteome has great potential as the next-generation biomarker. Firstly, urine is not regulated by the body's homeostatic mechanism and can sensitively reflect the early physiological or pathological changes of the body, which can play an important role in the early diagnosis of diseases[14]. Secondly, urine is easy to collect, preserve, and analyze. Urinary proteins can be directly bound to a PVDF membrane for long-term cryopreservation[15]. The above characteristics of urine determine that it can be a very promising biomarker and can play a more important role in the study of physiology and pathophysiology to the greatest extent.

In the study of urinary proteomics, using animal models can conveniently control variables and minimize the interference of related factors, enabling sample collection in the very early stage of disease occurrence and development[16][17]. The results of many animal model studies have shown that the urinary proteome can detect diseases earlier than blood, symptoms, signs, imaging, and

pathology. Currently, the urinary proteome has been studied in many diseases of various organs such as the liver, kidney, brain, and lung, such as tumors, degenerative diseases, and inflammations, and has verified the potential of the urinary proteome in early diagnosis[18][13][19][20][21][22][23].

Urinary proteomics has been applied to the study of many human diseases, and in the process of searching for biomarkers for the early diagnosis of certain diseases, urinary proteomics has shown great potential and has broad prospects in future clinical applications. Rubio - Sánchez R et al.[24] first studied the microorganisms in the vaginal secretions and urine of patients with trichomoniasis vaginalis infection to search for volatile biomarkers and found that volatile compounds such as 2 - octen - 1 - ol and 3 - nonanone could be used as biomarkers in urine samples, and 3 - hydroxy - 2,4,4 - trimethylpentyl - 2 - methylpropionate found in vaginal secretions was highly correlated with the urine of patients with trichomoniasis. In addition, searching for changes in the urinary proteome is an important method for the early diagnosis of urinary system and some systemic malignant tumors. It has shown great promise in the study of bladder cancer. Many potential markers of transitional cell carcinoma have been found in the past, such as hemoglobin, tumor - associated trypsin inhibitor, soluble cadherin E, and hyaluronidase, and some of them have been used clinically[25].

Therefore, this study conducted urinary proteomics analysis on people without the disease but with a family history of FTD and healthy people to explore whether there are differences in urinary biomarkers and biological pathways between those not identified with FTD under existing diagnostic methods and healthy people, aiming to promote the earlier diagnosis of FTD and provide a basis and clues for the prevention and treatment of FTD.

2 Materials and Methods

2.1 Urine Sample Collection

This study was approved by the Ethics Committee of Xuanwu Hospital (Clinical Research Review [2023]036). All participants provided written informed consent. The participants were from Xuanwu Hospital, and the sample collection time was August 26, 2023. The participant samples included 6 samples from unaffected individuals with a family history of FTD and 1 sample from an FTD patient. The collected urine samples were stored at - 80°C. The healthy samples were from a preprint study on COVID - 19, with a total of 11 samples[26].

Table 1 Sample information and statistical results

Sample Information		Healthy (n=11)	Unaffected Individuals with a Family History of FTD (n = 6)	FTD Patient (n = 1)
Age	Mean	42.9±6.5	43.3±9.7	59±0
	Age Range	34-56	33-61	59

2.2 Urine Sample Processing

Urinary proteins extraction: After thawing the urine samples, centrifuge at 12000 × g and 4°C for 20 minutes, and transfer the supernatant to a new EP tube. Then add dithiothreitol solution (DTT, Sigma) to a final concentration of 20 mM, heat in a metal bath at 37°C for 60 minutes, and cool to room temperature. Then add iodoacetamide (IAA, Sigma) to a final concentration of 50 mM, and place in the dark at room temperature for 40 minutes. Add four times the volume of absolute ethanol, mix well, and precipitate in a -20°C refrigerator for 24 hours. The next day, centrifuge the mixture at 12000 × g and 4°C for 30 minutes, discard the supernatant, and resuspend the protein precipitate in lysis buffer (containing 8 mol/L urea, 2 mol/L thiourea, 25 mmol/L DTT, 50 mmol/L). Centrifuge again at 12000 × g and 4°C for 30 minutes, and retain the supernatant. Measure the protein concentration using the Bradford method.

Protease digestion: Take 100 μg of the urinary proteins sample into an EP tube, add 25 mmol/L NH4HCO3 solution to make the total volume 200 μL as a sample for standby. Next, wash the membrane of the 10 kDa ultrafiltration tube (Pall, Port Washington, NY, USA): first add 200 μL of UA solution (8 mol/L urea, 0.1 mol/L Tris - HCl, pH 8.5), centrifuge at 14000 × g and 18°C for 10 minutes, and repeat twice. Add the above - prepared sample to the ultrafiltration tube and centrifuge at 14000 × g and 18°C for 40 minutes. Then add 200 μL of UA solution and centrifuge at 14000 × g and 18°C for 30 minutes, and repeat twice. Subsequently, add 25 mmol/L NH4HCO3 solution and centrifuge at 14000 × g and 18°C for 30 minutes, and repeat twice. Finally, add trypsin (Trypsin Gold, Promega, Fitchburg, WI, USA) at a ratio of trypsin:protein = 1:50 and incubate overnight at 37°C. After overnight incubation, centrifuge to collect the digested filtrate, perform desalting treatment through an Oasis HLB solid - phase extraction column, and vacuum - dry to obtain peptide lyophilization, which is stored at - 80°C.

2.3 LC - MS/MS Tandem Mass Spectrometry Analysis

Reconstitute the peptide with 0.1% formic acid water and dilute the peptide concentration to 0.5 μg/μL. Separate the peptides through a Thermo ESAY - Nlc1200 liquid chromatography system with the parameter settings as follows: elution time of 90 minutes and elution gradient (A phase: 0.1% formic acid; B phase: 80% acetonitrile). Repeat data - dependent mass spectrometry data acquisition on the separated peptides three times through an Orbitrap Fusion Lumos Tribird mass spectrometer using the data - independent acquisition mode.

2.4 Database Search

Use the Spectronaut Pulsar (Biognosys AG, Switzerland) software to search the database of the raw files acquired by the mass spectrometer and compare them with the SwissProt Human database. Calculate the peptide abundance by adding the peak areas of the respective fragment ions in MS2. Calculate the protein abundance by adding the abundances of the respective peptides.

2.5 Statistical and Bioinformatics Analysis

Each sample was technically repeated three times, and the average value of the obtained data was used for statistical analysis. This experiment performed a group comparison between the FTD family history unaffected group and the healthy group to screen for

differential proteins. The screening conditions for differential proteins were as follows: the fold change (FC) between groups ≥ 2 or ≤ 0.5, and the P value of the two - tailed unpaired t - test analysis < 0.01. The screened differential proteins were queried for their names and functions through the Uniprot website (https://www.uniprot.org), and the biological function enrichment analysis was performed through the DAVID database (https://david.ncifcrf.gov). The reported literature was retrieved in the Pubmed database (https://pubmed.ncbi.nlm.nih.gov) to analyze the functions of the differential proteins.

3 Results and Discussion

3.1 Comparison of Urinary Proteomes between the FTD Family History Unaffected Group and the Healthy Group

3.1.1 Differential proteins

The urinary proteins of the FTD family history unaffected group and the healthy group were compared, and the screening conditions for differential proteins were as follows: the fold change FC value (the ratio of the number of proteins identified in the urine samples of healthy people to that in the urine samples of individuals with a family history of FTD) ≥ 2 or ≤ 0.5, and the P value of the two - tailed unpaired t - test < 0.01. The results showed that compared with the healthy group, a total of 387 differential proteins were identified in the FTD family history unaffected group, of which 374 were downregulated and 13 were upregulated. The differential proteins were arranged in ascending order of FC, and detailed information was listed in Appendix Table 1. The information of the top 20 downregulated proteins and the 13 upregulated proteins among the differential proteins is listed in Table 2.

Table 2 Information of the Top 20 Downregulated Proteins and the 13 Upregulated Proteins among the Differential Proteins Screened with FC ≥ 2.0 or ≤ 0.5 and P < 0.01 between the FTD Family History Unaffected Group and the Healthy Group

Uniprot ID	Protein names	Fold change	P value	Trend
P05106	Integrin beta-3	0.000	0.000	↓
P30486	HLA class I histocompatibility antigen, B alpha chain	0.000	0.006	↓
Q13467	Frizzled-5	0.000	0.000	↓
Q13705	Activin receptor type-2B	0.000	0.002	↓
Q6UXH9	Inactive serine protease PAMR1	0.000	0.000	↓
Q7Z4P5	Growth/differentiation factor 7	0.000	0.003	↓
Q9NQ34	Transmembrane protein 9B	0.000	0.001	↓
Q9NRR1	Cytokine-like protein 1	0.000	0.001	↓
P26012	Integrin beta-8	0.001	0.000	↓
A0A0C4DH35	Probable non-functional immunoglobulin heavy variable 3-35	0.003	0.000	↓

P01782	Immunoglobulin heavy variable 3-9	0.003	0.000	↓
A0A087WW87	Immunoglobulin kappa variable 2-40	0.004	0.002	↓
Q86UP0	Cadherin-24	0.004	0.003	↓
P23434	Glycine cleavage system H protein, mitochondrial	0.005	0.000	↓
O75581	Low-density lipoprotein receptor-related protein 6	0.015	0.004	↓
P0DP01	Immunoglobulin heavy variable 1-8	0.015	0.000	↓
Q01628	Interferon-induced transmembrane protein 3	0.015	0.000	↓
Q7KYR7	Butyrophilin subfamily 2 member A1	0.018	0.000	↓
P35556	Fibrillin-2 [Cleaved into: Placensin]	0.018	0.006	↓
O75015	Low affinity immunoglobulin gamma Fc region receptor III-B	0.022	0.001	↓
Q9H008	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	2.036	0.005	↑
O75368	Adapter SH3BGRL	2.048	0.003	↑
Q9BRT3	Migration and invasion enhancer 1	2.182	0.005	↑
P20339	Ras-related protein Rab-5A	2.274	0.001	↑
Q9Y3B8	Oligoribonuclease, mitochondrial	2.672	0.003	↑
P35475	Alpha-L-iduronidase	2.756	0.002	↑
O15305	Phosphomannomutase 2	2.941	0.008	↑
Q8N5J2	Ubiquitin carboxyl-terminal hydrolase MINDY-1	4.962	0.009	↑
Q9BXF6	Rab11 family-interacting protein 5	5.485	0.009	↑
Q9BXD5	N-acetylneuraminate lyase	8.892	0.008	↑
Q2TAA2	Isoamyl acetate-hydrolyzing esterase 1 homolog	11.306	0.002	↑
P62857	Small ribosomal subunit protein eS28	18.283	0.007	↑
P09493	Tropomyosin alpha-1 chain	1240.937	0.009	↑

3.1.2 Random grouping

The samples of individuals with a family history of FTD (n = 6) and healthy samples (n = 11) were randomly divided into two groups, with a total of 12376 grouping combinations. Among all the random combination types, the average number of differential proteins in all random times was calculated as 14.49 under the same screening conditions. These results indicate that the randomly generated differential proteins were 14.49, and the credibility of screening differential proteins was 96.3%. This shows that the

changes in the urinary proteome of the FTD family history unaffected group relative to the healthy group were very significant.

3.1.3 Functional analysis of differential proteins

The identified differential proteins were retrieved in the Pubmed database for literature.

Among them, there were 8 differential proteins with the largest downregulation amplitude and a change of "from presence to absence", namely Integrin beta - 3, HLA class I histocompatibility antigen (B alpha chain), Frizzled - 5, Activin receptor type - 2B, Inactive serine protease PAMR1, Growth/differentiation factor 7, Transmembrane protein 9B, and Cytokine - like protein 1. The proteins that have been proven by existing literature to be related to neurological diseases and the physiological processes of the nervous system are as follows:

① Integrin beta - 3: Neuroinflammation involves cytokine release, astrocyte reactivity, and migration. Neuronal Thy - 1 can promote astrocyte migration by binding to Integrin beta - 3 and syndecan - 4 [27].

② Frizzled - 5: Frizzled - 5 is a receptor for Wnt proteins (WNT2, WNT10B, WNT5A). Studies have found that Frizzled 5 is continuously and cell - autonomously required for the survival of adult parafascicular nucleus neurons, and the defect in the Wnt - Frizzled signaling pathway may be the cause of neuronal loss in degenerative central nervous system diseases[28] .

③ Activin receptor type - 2B: During the development of the chick ciliary ganglion, researchers found that the signaling of activin receptor 2B plays an important role in the regulation of cell death during nervous system development. The downregulation of its expression leads to the dysregulation of neuronal apoptosis, affects the programmed cell death of neurons in vivo, and hinders the differentiation of choroidal neurons[29].

④ Growth/differentiation factor 7 (GDF7): Cerebellar granule neurons play a crucial role in cerebellar function. Rhombic lip cells express many granule neuron markers, while the dorsal midline cells adjacent to the rhombic lip cells express Bmp6, Bmp7 and GDF7. These three genes encode peptide growth factors of the bone morphogenetic protein (BMP) family. These BMPs, including GDF7, induce the expression of granule neuron markers in cultured neural tissues[30].

Among the 13 upregulated differential proteins, Tropomyosin alpha - 1 chain was upregulated nearly 1241 times in the FTD family history unaffected group, but there is currently no report on its association with frontotemporal dementia or neurological diseases. In addition, Rab11 family - interacting protein 5 (FC = 5.485, P = 0.009). Studies have shown that Rab11 mainly plays a key role in recycling various proteins including receptors important for signal transduction, thereby maintaining the functional activity of nerve cells. Rab11 family - interacting protein has a Rab - binding domain and mediates GTP - dependent interaction with Rab GTPases [31]; Researchers have observed a decrease in Rab11 expression in some neurodegenerative diseases, suggesting that Rab11 and its related proteins may be potential targets for therapeutic intervention[32] . Additionally, Ubiquitin carboxyl - terminal hydrolase MINDY - 1 (FC = 4.962, P = 0.009) is a protein that is significantly expressed in brain cells. Its level is related to the degree of neuronal brain injury and the disruption of the blood - brain barrier and can be detected in cerebrospinal fluid or blood in the acute phase of brain injury, indicating nervous system damage[33][34] . Finally, Alpha - L - iduronidase (IDUA) (FC = 2.756, P =

0.005) may interact with microtubule - bound motor proteins and be transported along microtubules. This transport mode can ensure efficient axonal - dendritic transport of IDUA within neurons, and neuronal injury may lead to the release of IDUA from neurons[35].

It is worth mentioning that Progranulin (PGRN) (FC = 0.284, P = 0.001) was found among the screened differential proteins. PGRN is encoded by the GRN gene and is a secreted protein that realizes its function through cell - cell signaling and signal transduction and is related to cell proliferation, wound repair, and growth factors. In many existing studies, it has been shown that GRN mutations can lead to PGRN haploinsufficiency and ultimately result in FTD, and it is considered an effective biomarker [8][36][37]. Moreover, GRN mutations may affect the normal function of PGRN by reducing its expression level, secretion, and affecting its subcellular localization[38].

In addition, there are other proteins that have been reported to be related to FTD or neurological diseases. The elevation of the level of Vascular endothelial growth factor A, long form (L-VEGF) (FC = 0.225, P = 0.004) is associated with neurogenesis, learning and neuroprotective effects. However, it may also increase vascular permeability, and L-VEGF is neurotoxic at high concentrations[39]. Lipopolysaccharide - induced tumor necrosis factor - alpha factor (FC = 0.225, P = 0.007) can activate microglia, promote inflammatory responses, and is related to neuronal death and cognitive decline. Studies have found that PGRN deficiency can lead to overactivation of NF - κB and increased TNF - α signaling in microglia, which in turn leads to excessive excitation of medium spiny neurons in the nucleus accumbens and obsessive - compulsive - like behaviors[40]. TGF - beta receptor type - 2 (TGFR - 2) (FC = 0.378, P = 0.005) has been reported to have an increased level in the cerebrospinal fluid of FTD patients. We also found the change of Metalloproteinase inhibitor 3 (TIMP - 3) (FC = 0.052, P = 0.004). Although there is currently no report on the relationship between TIMP - 3 and neurological diseases, studies have shown that the levels of its family members TIMP - 1 and TIMP - 2 change in FTD patients[41][42].

Our study shows that among the differential proteins of the FTD family history unaffected group compared with the healthy group, a large number of significantly changed proteins have been reported to be related to FTD or the nervous system. In addition, there are some differential proteins with very significant changes that have not been studied in relation to FTD or the nervous system. Perhaps further research on the functions of these proteins in FTD or the nervous system can be carried out in order to find biomarkers for the early screening of FTD or potential drug targets.

3.1.4 Enrichment Analysis of Biological Processes of Differential Proteins

The DAVID database was used to perform biological process (BP) enrichment analysis on the 387 differential proteins identified by group analysis. The results showed that a total of 139 BP pathways were significantly enriched ($P < 0.01$), and the detailed information is listed in Appendix Table 2.

The three biological pathways with the lowest P values enriched were cell adhesion, heterophilic cell - cell adhesion via plasma membrane cell adhesion molecules, and cell - cell adhesion, all of which were related to adhesion. Cell adhesion molecules (CAMs) can promote the interaction between leukocytes and endothelial cells, allowing leukocytes to enter the brain and trigger an

inflammatory response. On the other hand, some CAMs can promote the phagocytosis of microglia and clear pathological products such as Aβ. However, overactivated microglia may also release inflammatory factors and aggravate nerve damage[43].

At the same time, we enriched many pathways directly related to neurons and the nervous system, as shown in Figure 1a. Many studies have shown that glial cells and microglia are the key pathogenic factors of FTD[44][45]. Glial cells include various types such as astrocytes and oligodendrocytes. They provide support and protection for neurons and participate in the development, repair, and immune response of the nervous system. Astrocytes may participate in the development of FTD by releasing inflammatory factors and may also be involved in the aggregation process of abnormal proteins, further leading to neuronal damage and death[46][47]. Microglia, as the resident immune cells in the central nervous system, play a key role in neuroinflammation and neurodegenerative diseases. Moreover, microglia also play a role in synaptic pruning, which is the process of eliminating redundant synapses in the healthy developing brain. Previous studies have found that microglial proliferation and astrocyte increase usually occur in the brains of FTD patients[44]. In addition, the brain histology of FTD patients shows microvacuolar degeneration in the affected cortex, neuronal loss, partial neuronal swelling, white matter demyelination, and glial cell proliferation. Some basal ganglia and substantia nigra have severe neuronal loss, proving that the biological pathways we enriched are indeed related to the occurrence and development of FTD[48].

Among these biological processes, there are also many related to inflammation and immunity, as shown in Figure 1b. Neuronal dysfunction/death, abnormal protein accumulation, and activation of the central immune system are the main factors in the pathological progression of FTD. Abnormal protein conformation and accumulation will activate the immune system, leading to neuroinflammation[49]. White matter hyperintensity usually reflects the abnormality of brain blood vessels and nerve tissues. In FTD patients, severe white matter hyperintensity in the frontal lobe has been found. This white matter hyperintensity is related to extensive white matter demyelination and microglial dystrophy. Since microglia are immune cells in the nervous system, their dystrophy may lead to immune dysfunction and further aggravate neuroinflammation. Therefore, neuroinflammation may play an important role in white matter lesions[50].

In addition, cerebral microbleeds are quite common in neurocognitive disorders. It has been found that cerebral microbleeds can increase the risk of dementia including Alzheimer's disease, possibly by inducing spine elimination through fibrinogen, thereby activating neuroinflammatory and neurodegenerative processes in microglia. This explains the occurrence of biological processes such as blood coagulation[51].

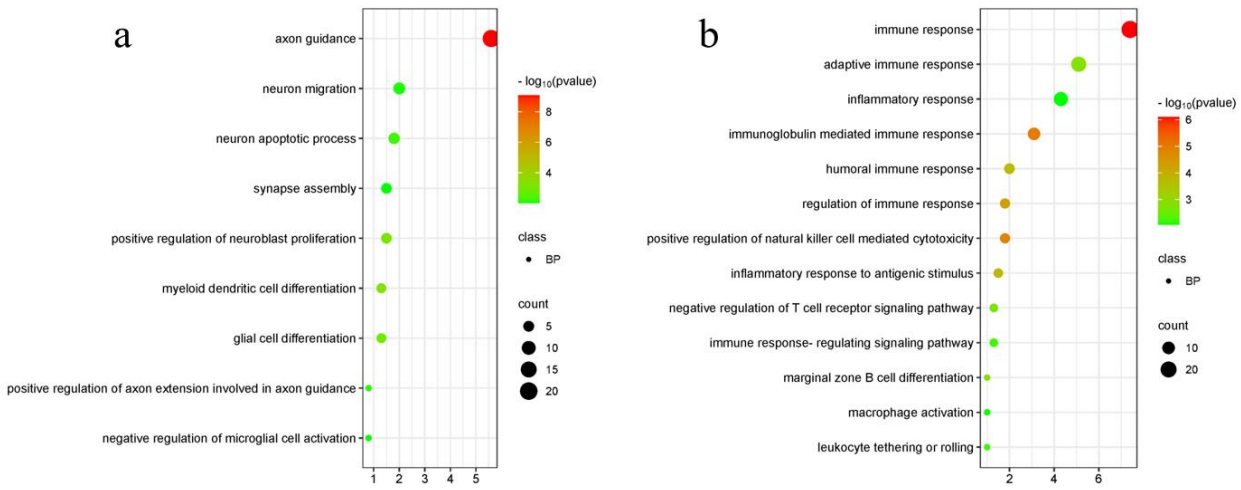

Figure 1 Biological Processes Enriched by Differential Proteins in the FTD Family History Unaffected Group and the Healthy Group. a. Neuron and Nervous System - Related Pathways; b. Inflammation and Immunity - Related Pathways.

3.2 Cluster Analysis of Samples from Unaffected Individuals with a Family History of FTD, FTD Patients, and Healthy Individuals

Cluster analysis was performed on the total proteins of samples from unaffected individuals with a family history of FTD, FTD patients, and healthy individuals (samples 1 - 6 are from unaffected individuals with a family history of FTD, 7 is an FTD patient, and H1 - H11 are healthy individuals). Proteins with the same or similar expression patterns were clustered to obtain a total protein cluster analysis diagram (Figure 2). As can be seen from the figure, only one healthy sample (H7) was not separated from the unaffected individuals with a family history of FTD among all samples, further proving that the total proteins of the unaffected individuals with a family history of FTD are different from those of healthy individuals.

We also noticed an interesting phenomenon. In the cluster analysis diagram, the aggregation of members of the same family occurred (samples 1, 2, 3, 4, and 6 are from the same family, and samples 5 and 7 are from another family). A similar phenomenon also occurred in the study of Parkinson's disease by Mann et al.[52]. However, the two families of FTD samples in this experiment are from different regions (Liaoning Province, China; Hebei Province, China). Previous studies have shown that the urinary proteomes of people in different regions of China have significant regional differences. Region has a great impact on human urinary proteins. Therefore, the family aggregation phenomenon needs further exploration[53].

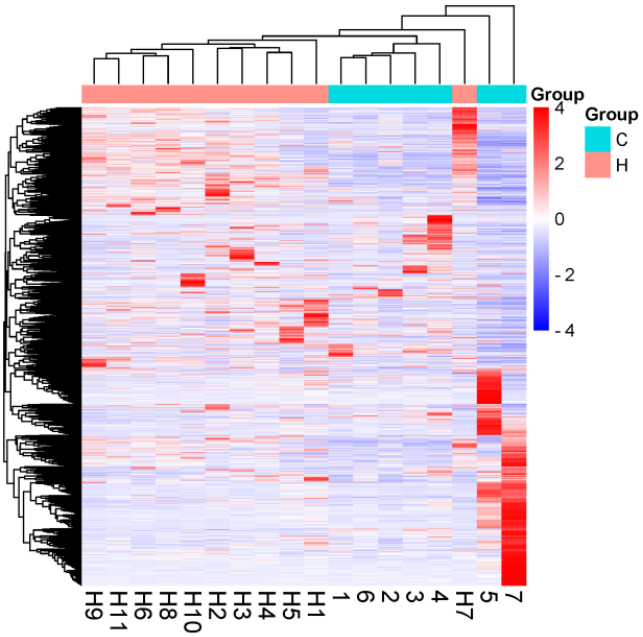

Figure 2 Cluster Analysis of Total Proteins in Samples from Unaffected Individuals with a Family History of FTD, FTD Patients, and Healthy Individuals (1 - 6 are Unaffected Individuals with a Family History of FTD, 7 is an FTD Patient, and H1 - H11 are Healthy Individuals)

4 Conclusion

This study conducted urinary proteomics analysis on the population of unaffected individuals with a family history of FTD and the healthy population and found that urinary proteomics can clearly distinguish unaffected individuals with a family history of FTD from healthy individuals. Unaffected individuals with a family history of FTD have proteins and biological pathways that are different from those of healthy people, and many of these differential proteins and biological pathways have been reported to be directly related to FTD or related to neurological diseases and the nervous system. Other proteins and pathways that have not been studied in the direction of FTD or neurological diseases are also worthy of further research to explore their roles in the nervous system.

These findings suggest the potential of urinary proteomics in searching for potential FTD patient populations and exploring the mechanisms related to the early occurrence and development of FTD, providing a new perspective for the prevention and early diagnosis of FTD, which is worthy of further long - term research by researchers.

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