Serum biomarkers research of tourette's syndrome children using proteomics techniques–a pilot study

WenXiang He
Children's Hospital affiliated to Xi'an Jiaotong University https://orcid.org/0000-0002-9324-4105
YanNi Chen (Chenyannichil@163.com)
Children's Hospital affiliated to Xi'an Jiaotong University
Duan Wang
Children's Hospital affiliated to Xi'an Jiaotong University
HaiQing Zhang
Children's Hospital affiliated to Xi'an Jiaotong University
Hui Zhang
Department of Pediatrics,Affiliation Hospital of Shanxi University of Traditional Chinese Medicine
YanFang Ma
Department of Pediatrics,Affiliation Hospital of Shanxi University of Traditional Chinese Medicine

Research

Keywords: Tourette's syndrome, proteomics, Serum biomarkers

Posted Date: September 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-69262/v1

Abstract

Purpose:This study used proteomics to analyze the changes in serum proteomics between tourette's syndrome (TS) children and healthy children in order to find serum biomarkers that can distinguish TS children from healthy children.

Experimental design: We analyzed the serum proteome of 60 TS children and 30 healthy controls children using magnetic bead-based weak cation exchange (MB-WCX) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). Next, we identified candidate biomarkers using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Candidate biomarkers were then validated using ELISA and western blotting.

Results: 59 peaks were identified and the expression fold changes of seven peaks in the two groups were greater than 1.9. Two peaks (m/z: 6443.34 Da; m/z: 6642.05 Da;) tended to be upregulated, whilefive peaks (m/z: 863.13 Da; m/z: 2175.98 Da; m/z: 2191.841 Da; m/z: 2277.19 Da;m/z: 2293.11 Da) tended to be down-regulated in TS group. The peak for a 2191.84 Da peptide was identified as FGA (Isoform 1 of the Fibrinogen alpha chain precursor, FGA);The peak for a 2175.98 Da peptide was identified as PKM2 (Isoform M2 of Pyruvate kinase isozymes,PKM2);The peak for a 2277.19 Da peptide was identified as GAPDH (Glyceraldehyde-3-phosphate dehydrogenase,GAPDH);The peak for a 863.13 Da peptide was identified as PROC (Vitamin K-dependent protein C,PROC).Enzyme-linked immunosorbent assay (ELISA) analyses revealed that the expression of FGA and PKM2 were significantly higher in TS children than healthy controls children.

Conclusion: FGA and PKM2 may be potential serum biomarkers to distinguish TS children from healthy children.

1. Introduction

Tourette's syndrome (TS) is severe hierarchy of Tic disorders (TD) which is a kind of neuropsychiatric disease. TS start from childhood and take both multiple motor and one or more vocal tics have been present at some time during the illness, although not necessarily concurrently. It is a group of motor disorders with unknown causes, mainly manifested as involuntary, repeated, rapid and aimless muscle motor twitch and/or vocal twitch in one or more parts. The prevalence rate is increasing year by year, and the estimated prevalence of TD ranges from 3 to 8 per 1,000 in school-age children[1]. Behavioral comorbidities often associated with TS account for up to 50-90% of TS patients and may include attention deficit hyperactivity disorder(ADHD), obsessive-compulsive behavior(OCB),anxiety, and depression[2] and it is becoming one of the common serious chronic diseases that endanger children's health[3]. Because of the comorbidities, TS clinical picture interferes with normal social functioning. In recent years, a number of studies have found that TS has a highly hereditary and complex polygenic background[4].The abnormal function of the cortex-striatum-thalamus-cortex circuit is the basis of TS[5]. Imbalance of neurotransmitters such as dopamine, noradrenaline, and 5-hydroxytrytamine in the circuit

are the main causes of TS in recent research works[6], infectious and immune factors[7]and sociopsychological factors[8] are closely related to the occurrence of TS. However, so far, the exact etiology and pathogenesis of the disease remain unclear. So none was effective treatment, involving in neuroleptics, adrenergic agonists, dopamine agonists, behavioral techniques, and botulinum toxin untill now[9]. The diagnosis of the disease currently depends on the patient's clinical manifestations for descriptive diagnosis. Due to the lack of objective diagnostic indicators in clinical work, some TS patients cannot be diagnosed early, or even missed or misdiagnosed. Therefore, the clinically is an urgent need to find the exactly pathogenesis and further diagnosis and treatment method.

As an important method in the post-gene era, proteomics technology has become a very important research platform in the field of molecular medicine because of its fast, high-throughput, high sensitivity, and easy operation[10]. Proteomic technologies enable the large-scale and in-depth investigation of proteins, in particular, the screening of potential biomarkers in complex biological matrices in a high-throughput manner. To date, proteomics has become a fundamental component for biological study and is recognized as a key method for novel biomarker discovery and personalized therapy[11], Especially after the launch of the human plasma proteome project that was proposed at Human Proteome Organisation 2010 in Sydney[12]. As indicators of pathogenic processes and pharmacologic responses to a therapeutic intervention, biomarkers enable us to accurately predict pathophysiology as well as understand how it is altered by treatments. The serum of blood in human physiology implies that it should be a universal reflection of an individual’s state or phenotype. Pathophysiological changes in the body at any time can be reflected in the serum. Clinically, the detection of physiological indicators or parameters in the serum/plasma has always been one of the most commonly used auxiliary diagnostic and monitor therapy methods. It is more practical to take serum proteins involved in many physiological and pathological processes as research objects. Serum proteomics is currently the most commonly used method for screening disease markers. Serum proteomics studies all the proteins expressed in the serum of selected populations, and on the basis of establishing a normal protein expression profile, looking for its differential protein spots and identifies disease-related proteins. To further study its structure and function, and open up new avenues for studying the pathophysiology of major diseases, specific markers for early diagnosis, and drug targets[13]

In recent years, the proteomics method has made great progress in the field of nervous system disease research, and the study of biological targets of nervous system diseases has become a hot spot today. Yang et al[14],Used proteomics to analyze the serum proteins of children with ASD and found that there are 8 differentially expressed proteins in the serum of children with ASD, which not only revealed the etiology of ASD from the perspective of serum proteomics, but also verified these 8 This differential protein may be a potential biomarker for the diagnosis of ASD.Jiang et al[15],Used SELDI-TOF-MS technology to study the serum of healthy controls and patients with schizophrenia to find 6 marker proteins with significant differences in expression. This technology established an artificial neural network model for schizophrenia. Shen et al[16], Used MALDI-TOF-MS technique to compare the differential expression of serum proteins between NTD pregnant women and normal pregnant women,

and found that the sensitivity and specificity of the three proteins in diagnosing NTD could reach 96% and 90% by protein fingerprint software. Bao et al[17],Conducted iTRAQ marking and analysis on plasma samples collected from patients with severe brain injury with chronic consciousness impairment in different time periods and healthy people, and identified 32 proteins with significant changes after brain injury, and these differential proteins are mainly involved in complement system pathways. The last, an iTRAQ-MS study of AD and thecontrol plasma proteome showed that Complement 4a, an inflammatory-related protein, is upregulated in the plasma of AD patients. This was further validated by immunoblot and ELISA[18].Additionally, YKL-40 was shown to be potential biomarker of AD through LC-MS/MS-based proteomic profiling method, and it was validated in another cohort[19] .

In this study, serum proteomic profiling in children with TS and healthy controls were conducted by MB-WCX purification combined with MALDI-TOF-MS. ClinProTools software was used to compare the generated serum proteomic profiles. The significant differentially expressed peaks between TS group and healthy control group could be considered as potential serum biomarkers and were sequenced and further identified. The potential serum biomarkers were then analyzed by bioinformatics and validated using ELISA. The validated serum biomarkers may supply a more useful and reliable method assisting the diagnosis of TS.

2. Experimental Section

2.1. Participants

A total of 90 Chinese Han children were used in this study, including 60 in the TS group and 30 in the healthy control group. Children in the TS group were recruited from Xi'an Children's Hospital in Shaanxi Province, China, and all children with TS were examined by a pediatric psychiatrist. Inclusion criteria: (1)The TS group all met the diagnostic criteria for Diagnostic and statistical manual of mental disorders(DSM5)[1]tics in the United States;(2) Children under 18 years old, not limited to male and female;(3)No other neuropsychiatric diseases;(4) Have not taken any drugs that affect neurotransmitters. There was no significant difference in age and gender among all recruiters.

The study was approved by the Ethics Committee of Xi'an Children's Hospital. After understanding the potential risks of the study, the guardians of all participants have signed written informed consent. All experiments were performed in accordance with the approved guidelines.

A fasting blood draw was performed on all TS and control subjects between 8 and 10 a.m. Blood samples were collected in vacuum blood collection tubes lacking heparin and centrifuged at 3000 g for 10 min at 4°C to separate the serum. The supernatant was divided into 100 μL aliquots and stored at -80°C until processing.

2.2. MALDI-TOF MS Analysis

MB-WCX Profiling Kit (Bruker Daltonics), based on magnetic bead technology utilizing the interaction mechanisms of cation exchange, is designed for sophisticated biomarker profiling studies and was used for proteomics or peptidome separation of serum samples according to the standard protocol by Wang et al[20].A total of 90 serum samples were fractionated. Magnetic beads were then thoroughly mixed on a vortex device for at least 1 min, before 10μL of magnetic beads with 10μL binding buffer was added to a standard thin wall PCR-tube and mixed by pipetting up and down. Next, 5μL serum was added to the solution and mixed by pipetting up and down five times. After 5 min of incubation, the tube was placed into the magnetic separator and the beads were automatedly collected at the wall of the tube for 1 min. The supernatant was collected carefully until it was clear from magnetic beads, and then washed three times with 100μL washing buffer. The peptide fraction was eluted from the magnetic beads with 5μL of elution solution and 5μL of stabilization buffer. The eluted peptides were spotted onto cleaned MALDI-TOF-MS targets with 1μL of matrix consisting of 4 mg mL^{-1} a-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile. Then, 0.5% trifluoroacetic acid was added twice to the MALDI AnchorChip surface. To ensure reliability of the method,all samples were spotted in triplicate. Instrument calibration parameters were determined using standard peptide and protein mixtures (Bruker).

2.3. ClinProTools Analysis

Immediately after, all targets were analyzed by calibrated Autoflex III MALDI-TOF MS (Bruker) using an optimized protocol of FlexControl version 3.0 software (Bruker). The ClinProTools software (Bruker Daltonik) has been used for all data interpretation steps, which include baseline correction, smoothing, peak detection and alignment, as well as further analysis.The whole data pretreatment has been completed using default settings and was performed automatically. The pretreated data have been used for visualization and statistical analysis in ClinProTools. Peak statistics has been performed by means of a Welch's t-test.Use FlexAnalysis3.0 peak software and ClinproTools statistical software to analyze protein peaks.Genetic algorithms (GA), based on evolutionary survival, were used for model analysis and were applied to select the combinations of peaks that perform best in separating the classes. It is suited for a population with a large variety of peak combinations. The fifittest peak combinations were selected and the less capable abandoned. This procedure aims for the most favorable class separation with high variance between classes to optimize a cost function.The difference peak standard $P < 0.05$ was selected, and the multiple of the difference was> 1.9. The area under the working characteristic curve (AUC) of the subjects was> 0.7.

2.4 Peptide identification

After completing the statistical analysis, selected peptides or proteins biomarkers were purified and separated using Nano Aquity UPLC C18 beads and serially eluted with 5% and 95% acetonitrile.Use Q-Exactive HF mass spectrometer (Thermo Sceientific) for mass spectrometry analysis, the raw data of mass spectrometry analysis is RAW file, use software software Mascot 2.2 and Proteome Discover2.0 to search the database uniprot_human_fasta, identify the selected peptide or protein biomarker Thing.

2.5. ELISA

In the ELISA analysis, all serum samples were tested in a blinded fashion, standards and samples were run in triplicate.The concentrations of Isoform 1 of Fibrinogen alpha chain(FGA),Isoform M2 of Pyruvate kinase isozymes(PKM2), Tubulin beta chain(TUBB), 2 Glyceraldehyde-3-phosphate dehydrogenase(GAPDH), and Vitamin K-dependent protein C(PROC), quantifified using a Human FGA ELISA Kit ,Human PKM2 ELISA Kit,Human TUBB ELISA Kit,Human GAPDH ELISA Kit,and HumanPROC ELISA Kit.A standard curve was generated and used to determine the concentrations of FGA, PKM2,TUBB,GAPDHand PROC in the samples analyzed.

2.6. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). All data are shown as the mean ± standard deviation. A p-value below 0.05 was considered statistically significant. Students t-test.were used to compare differences between groups.

3. Results

3.1 Clinical Characteristics Analysis

Demographic features between TS group and healthy control group were similar.In the TS group, there were 52 male and 8 female subjects with an average age of 89.52±26.76 months.In the healthy control group, there were 26 male and 4 female subjects with an average age of 88.32±25.32 months.There were no statistically signifificant diffffferences between the groups with respect to the distribution of age and sex ($p > 0.05$).

3.2 Serum Proteomic Profifiles of Diffffferent Groups

To ensure reproducibility and stability of mass spectra, we analyzed all samples in triplicate and found closely reproducible peaks (Figure1A,B). MB-WCX and MALDI-TOF-MS were used to compare the proteomic profiles of the TS group and the control group. Fractionation of serum samples showed that TS (red) and control subjects (green) had proteomic profifiles from 0.8-10kDa (Figure 1).The differentially expressed peaks between the two groups were detected in this mass range. According to the serum proteomics analysis, only a few overlapping areas between TS group and healthy control group were found, indicating that the groups were accurately distinguished(Figure 2A,B).

3.3 Peaks Selection

TS group and healthy control group have 59 distinguishable differentially expressed protein peaks. Taking the multiples of peak intensity difference between groups (average ratio of peak intensity between groups)>1.9 as the significant difference, 7 protein peaks have significant differences ($P < 0.001$) and their AUC values are greater than 0.70;In these 7 protein peaks, the relative molecular masses were 6443.34 Da, and 6642.05 Da expression was up-regulated, the masses were 863.13 Da,2175.98 Da,2191.84 Da,

2277.19 Da and 2293.11 Da expressions was down-regulated(Table 1).Differential protein peaks and ROC curves are shown in Figure 3.

Table 1. Mean levels of 7 diffffferentially expressed proteins peaks in healthy control group and TS group.

Mass (m/z)	fold change	p-value	ROC area (AUC)	Expression
6443.34	2.46	< 0.001	0.831	Up
6642.05	3.24	< 0.001	0.861	Up
863.13	3.19	< 0.001	0.779	Down
2175.98	2.02	< 0.001	0.778	Down
2191.84	2.08	< 0.001	0.783	Down
2277.19	2.08	< 0.001	0.781	Down
2293.11	1.98	< 0.001	0.754	Down

3.4 Identification of serum differentially expressed proteins

The seven potential (m/z: 2191.84 Da, 2175.98 Da, 2277.19 Da, 863.13 Da, 6643.34 Da, 6642.05 Da, 2293.11 Da)serum biomarkers were further sequenced using LC-ESI-MS/MS and identified using the uniprot database. Among them, the proteins or peptides with Mass of 6633.34 Da, 6642.05 Da, and 2293.11 Da did not find any relevant proteins matching them during the mass spectrometry identification process. A total of four peptides were identified. MS/MS fragmentation of these four peptides identified the relevant peptide sequences listed in Table 2(Figures 4,5,6,7, Supporting Information).

Table 2. Sequence identification of four Serum peptide biomarkers for TS

Mass (m/z)	Peptide sequence	Identify
2191.84	TSSTSYNRGDSTFESKSYKMA	Isoform 1 of Fibrinogen alpha chain（FGA）
2175.98	RLAPITSDPTEATAVGAVEASFKC	Isoform M2 of Pyruvate kinase isozymes（PKM2）
2277.19	KWGDAGAEYVVESTGVFTTMEKA	Glyceraldehyde-3-phosphate dehydrogenase （GAPDH）
863.13	RLGEYDLRR	Vitamin K-dependent protein C（PROC）

3.5 ELISA verification results of serum differential proteins

To screen for serum biomarkers of TS and determine the expression level of FGA, PKM2, GAPDH, and PROC, serum concentrations were examined by ELISA in the 90 samples from TS group and healthy control group. Serum concentrations of FGA,PKM2, GAPDH, and PROC in the two groups are shown in

Table 3. All data are represented as mean ± SD. The statistical analysis was performed with Prism 5.0 software. The serum levels of FGA and PKM2 protein in children with TS group were significantly lower than those in healthy controls (P <0.05, Figures 8), which were basically consistent with the results of mass spectrometry analysis.

Table 3. Mean levels of differentially expressed proteins between TS group and health control group

Proteins	Normal	TS	p-value
FGA[μg/mL]Mean±SD	3223.20±470.39	2504.06±490.84	<0.0001
PKM2[pg/mL]Mean±SD	46.18±7.68	33.79±6.25	<0.0001
GAPDH[g/mL]Mean±SD	886.57±122.43	789.31±237.94	>0.05
PROC[pg/mL]Mean±SD	2.67±0.54	2.59±0.50	>0.05

4. Discussion

TS is a neuropsychiatric disorder that starts in childhood and is mainly manifested by tics[21].Strong evidence supports involvement of cortical–basal ganglia–thalamocortical circuits in the pathophysiology of tic disorders. Several neurotransmitters,including dopamine, glutamate,GABA, serotonin, acetylcholine, norepinephrine,endogenous cannabinoids, opioids, histamine, and adenosine, are active participants within these circuits and may be dynamic factors in the pathophysiology of tics. However, the etiology and pathogenesis of the disease have not been clarified so far, the clinical diagnosis lacks specific indicators, so that the disease cannot be diagnosed in a timely and accurate manner, which affects the prognosis; This study used proteomics techniques to study serum proteins in children with tic disorder and found that two proteins named FGA and PKM2 may be serum biomarkers that distinguish TS group from healthy children.

Isoform 1 of Fibrinogen alpha chain (FGA) is a subtype of fibrinogen. Fibrinogen is a glycoprotein found in the plasma of all vertebrates[22], which has the functions of regulating immune inflammatory response, enhancing blood clotting function and inhibiting tissue wound repair.As an acute inflammatory response protein, fibrinogen has a unique molecular structure that binds to integrins secreted by various immune cells and participates in the inflammatory response of the nervous system[23].Fibrinogen binds to CD11b/CD18 integrins, which are synthesized and secreted by various immune cells, widely stimulates inflammatory response signal of cells, promotes local production of inflammatory cytokines such as TNF- and IL-1β, and participates in regulating immune function, inducing adhesion, migration, chemotaxis and phagocytosis of immune cells, and strengthening host active immune defense function[24].In recent years, many studies have shown that tic disorder is related to immune response. Some researchers[25][26][27][28] at home and abroad have studied the cytokines in the serum of children with TS and found that some cytokines in the serum of children with TS are abnormal, and the concentration level is related to the severity of the disease. The level is positively correlated, suggesting

that the abnormality of cytokines is closely related to the occurrence of tics.The mechanism may be that the neuroendocrine - immune network is involved in the pathogenesis of TS.Children with TS are in the state of cellular immune activation, and cytokines may promote the occurrence of TS.Kawikova[29], Bos-Veneman[30], Landau[31] and other teams studied the expression of some immunoglobulins in children with TS and found that the occurrence of TS is closely related to the function of humoral immunity. The pathogenesis may be that the antigen and antibody combine to form an antigen-antibody complex, which activates cytokines, thereby triggering neurological damage, leading to the occurrence of TS.Our research found that FGA1 was significantly underexpressed in the serum of TS children. That is to say it is possible that FGA take role in immune response related to immunologic mechanism of TS. It is worth to do further research deeply about the role in TS immunologic mechanism. The result also indicates FGAcould be a potential serum marker for children with TS.

Isoform M2 of Pyruvate kinase isozymes (PKM2) is a subtype of Pyuvate kinase (PK) and the rate-limiting enzyme in the last step of the glycolysis process. It can decarboxylate phosphoenolpyruvate to pyruvate and produce ATP[32].PKM2 is mainly expressed in embryonic cells, tumor cells and other rapidly dividing and proliferating cells[33][].The Warburg effect in which PKM2 is involved is a peculiar glycolysis phenomenon. As a major metabolic pathway in the early development of brain nerves after birth, the Warburg effect is involved in axonal elongation, synapse generation and myelin process[34].The Warburg effect in which PKM2 participates is closely related to the occurrence of neurological diseases,The Afaf[35] study found that the content of pyruvate kinase in the plasma of autistic patients was significantly reduced compared with the control group, which reflected the decreased adaptability of the patients to impaired energy metabolism. Newington et al found that the Warburg effect can alleviate the process of Alzheimer's diseases(AD) by inhibiting the cell death mediated by amyloid β-mediated(Aβ)cell death in the early stage of AD.Some researchers have found that the activation of PI3K/Akt pathway in HD patients increases the lactate/pyruvate ratio in YAC128 mice and HD patients, and up-regulates the Warburg effect. This energy metabolism disorder accelerated apoptosis and accelerated the course of HD disease[36][37].Because the developmental brain tissue is dependent on energy supply, especially glucose metabolism, a slight lack of energy production may have a significant impact on neurons and glial[38].TS is a multi-causal disease of brain development disorders, impaired energy metabolism may be one of the causes of this multi-factor disease TS symptoms may worsen when tired, whether or not impaired PKM2 metabolism is related to this phenomenon requires further study. The result also indicates PKM2 could be a potential serum marker for children with TS.

Limitations

The limitation this research relates to the small sample size. Future research should expand the sample size so that there are enough samples to test the role of the FGA and PKM2 in TS children, and verify the quantity in TS serum .

This study uses proteomics technology to study the differential proteins between TS children and healthy children. Although two specific serum biomarkers (FGA and PKM2) were identified that might distinguish

TS group from healthy control group. However, the relationship between these markers and pathogenesis needs further deeper research. We need to further clarify the relationship between these serum markers and the pathogenesis of TS.

Abbreviations

TS
Tourette's syndrome
MB-WCX
Magnetic bead-based weak cation exchange
LC-ESI-MS/MS
Liquid chromatography-electrospray ionization-tandem mass spectrometry
ELISA
Enzyme-linked immunosorbent assay

Declarations

Funding

This work was supported by the Subject Innovation Team of Shaanxi University of Chinese Medicine (Grant No.2019-YL07),the National Natural Science Foundation of China Funded Project (Grant No. 81371900),the Science and Technology Research and Development Program of Shaanxi Province (Grant No. 2013SF2-009);the Supported by Basic Research Plan of Natural Science of Shaanxi Province(Grant No.2019JM4030),the Xi 'an Science and Technology Planning Project (Grant No.20YXYJ0006(4)),and the Key R & D program of Shaanxi Province(Grant No.S2019-YF-GXYB-0228).

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of Children's Hospital affiliated to Xi 'an Jiaotong University. Written informed consent was obtained from all subjects.

Conflict of interest

The authors have no conflicts of interest to disclose.

Contributions

HWX participated in the data collection, statistical analysis and data interpretation, manuscript drafting and critical revision of the manuscript.. WD,ZH, and MYF participated in the data collection.WD and ZHQ contributed to statistical analysis and data interpretation. WD participated in the design and execution of the study. CYN supervised the design and execution of the study, performed the final data analyses and

contributed to the critical revision of the manuscript. All authors contributed to the final version of the article.

References

1] American Psychiatric Association.Diagnostic and Statistical Manual of Mental Disorders(DSM5).5thed.Arlighton,VA：American Psychiatric Association,2013:8
2] Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P. An international perspective on Tourette syndrome: selected findings from 3,500 individuals in 22 countries. Dev Med Child Neurol. 2000;42(7):436-447. doi:10.1017/s0012162200000839
3] Brander G, Rydell M, Kuja-Halkola R, Fernández de la Cruz L, Lichtenstein L,Serlachius E,et al. Perinatal risk factors in Tourette's and chronic tic disorders: a total population sibling comparison study. Mol Psychiatry. 2018;23(5):1189-1197. doi:10.1038/mp.2017.31
4] Robertson MM, Eapen V, Singer HS, Martino D, Scharf JM,Paschou P,et al. Gilles de la Tourette syndrome. Nat Rev Dis Primers. 2017;3:16097. doi:10.1038/nrdp.2016.97.
5] Ganos C, Roessner V, Munchau A.The functional anatomy of Gilles de la Tourette syndrome. Neurosci. Biobehav. 2013. 37(6):1050-1062 .doi:10.1016/j.neubiorev.2012.11.004.
6] Hartmann A, Worbe Y. Tourette syndrome: clinical spectrum, mechanisms and personalized treatments. Curr Opin Neurol. 2018;31(4):504-509. doi:10.1097/WCO.0000000000000575.
7] Pagliaroli L, Vető B, Arányi T, Barta C. From Genetics to Epigenetics: New Perspectives in Tourette Syndrome Research. Front Neurosci. 2016;10:277.doi:10.3389/fnins.2016.00277
8] Paschou P, Müller-Vahl K. Editorial: The Neurobiology and Genetics of Gilles de la Tourette Syndrome: New Avenues through Large-Scale Collaborative Projects. Front Psychiatry. 2017;8:197. Published 2017 Oct 11. doi:10.3389/fpsyt.2017.00197.
9] Párraga HC, Harris KM, Párraga KL, Balen GM, Cruz C. An overview of the treatment of Tourette's disorder and tics. J Child Adolesc Psychopharmacol. 2010;20(4):249-262. doi:10.1089/cap.2010.0027.
10] Alvarez-Chaver P, Otero-Estévez O, Páez de la Cadena M, Rodríguez-Berrocal FJ, Martínez-Zorzano VS. Proteomics for discovery of candidate colorectal cancer biomarkers. World J Gastroenterol. 2014;20(14):3804-3824. doi:10.3748/wjg.v20.i14.3804.
11] Mallick P, Kuster B. Proteomics: a pragmatic perspective. Nat Biotechnol. 2010;28(7):695-709. doi:10.1038/nbt.1658.
12] Anderson NL, Anderson NG, Pearson TW, Borchers CH,Paulovich AG,Patterson SD,et al. A human proteome detection and quantitation project. Mol Cell Proteomics. 2009;8(5):883-886. doi:10.1074/mcp.R800015-MCP200.
13] Liao QL,Chen XD,Ding YQ.Advance in serum proteomics research and technology[J].CHINESE JOURNAL OF PATHOPHYSIOLOGY,2008,24(2):402-407. DOI:10.3321/j.issn:1000-4718.2008.02.045.
[14] Yang J, Chen YN, Xiong XF, Zhou XB,Han L,Ni L,et al. Peptidome Analysis Reveals Novel Serum Biomarkers for Children with Autism Spectrum Disorder in China. Proteomics Clin Appl.

2018;12(5):e1700164. doi:10.1002/prca.201700164.
[15] Jiang W.Application of Serum Protein Markers from Patients with Schizophrenia in Clinic.Luzhou, China: Luzhou Medical College, 2008.
[16] Shen G, He P, Du Y, Zhang S. Identification of Biomarkers by Proteomics for Prenatal Screening for Neural Tube Defects. Tohoku J Exp Med. 2016;238(2):123-129. doi:10.1620/tjem.238.123.
[17] Bao WX, He FP, Yu LH,Gao J, Meng FX,Ding YH,et al. Complement cascade on severe traumatic brain injury patients at the chronic unconscious stage: implication for pathogenesis. Expert Rev Mol Diagn. 2018;18(8):761-766. doi: 10.1080/ 14737159.2018.147 1985.
[18] Bennett S, Grant M, Creese AJ, Mangialasche F, Cecchetti R, Cooper HJ,et al.Plasma levels of complement 4a protein are increased in Alzheimer's disease. Alzheimer Dis Assoc Disord. 2012;26(4):329-334. doi:10.1097/WAD.0b013e318239dcbd
[19] Craig-Schapiro R, Perrin RJ, Roe CM,Xiong Cj,Carter D,Cairns NJ,et al. YKL-40: a novel prognostic fluid biomarker for p Alreclinicalzheimer's disease [published correction appears in Biol Psychiatry. 2011 Feb 15;69(4):389]. Biol Psychiatry. 2010;68(10):903-912. doi:10.1016/j.biopsych.2010.08.025.
[20] Wang D,HE L,Zhang J,Zhao XY, Xiong XF,He WX,et al.Screening of serum related proteins of different TCM syndromes with tic disorders by proteomic techniques.Chinese Journal of Woman and Child Health Research.2018,29(2):149-153. DOI: 10.3969/ j.issn.1673 -5293.2018.02.005.
[21] Neurology Group, Pediatrics Branch of Chinese Medical Association.Expert consensus on the diagnosis and treatment of tic disorders in children (2017 practical edition).Chinese Journal of Applied Clinical Pediatrics.2017,32(15):1137-1140.doi:10.3760/cma.j.issn.2095-42
8X20 17.15.005.
[22] Weisel JW, Litvinov RI. Fibrin Formation, Structure and Properties. Subcell Biochem. 2017;82:405-456. doi:10.1007/978-3-319-49674-0_13.
[23] TP U, DA S, L Z, et al. Identification of a novel recognition sequence for integrin alphaM beta2 within the gamma-chain of fibrinogen.[J]. Journal of Biological Chemistry, 1998,273(35): 22519-22527.
[24] Hsieh JY, Smith TD, Meli VS, Tran TN, Botvinick EL, Liu WF. Differential regulation of macrophage inflammatory activation by fibrin and fibrinogen. Acta Biomater. 2017;47:14-
doi:10.1016/j.actbio.2016.09.024.
[25] Liu Z,Ji JP,Chen H,Li J,Zhang Y,Kang JJ.Correlation study between T-lymphocyte subsets and emotion in children with Tourette syndrome.China Journal of Modern Medicine,2013,23(27):101-105.
[26] Li N,Du JJ,Zheng H,Ji WD.Study on relationship between Tourette's syndrome and ASO, IL-6, IL-8.Chinese Journal of Child Health Care,2013,21(7):688-690.
[27] Cheng YH, Zheng Y, He F, Yang JH,Li WB, Wang ML,et al. Detection of autoantibodies and increased concentrations of interleukins in plasma from patients with Tourette's syndrome. J Mol Neurosci. 2012;48(1):219-224. doi:10.1007/s12031-012-9811-8.
[28] Leckman JF,Katsovich L,Kawikova I,Lin HQ,Zhang HP,Krönig H,et al. Increased serum levels of interleukin-12 and tumor necrosis factor-alpha in Tourette's syndrome.Biol Psychiatry. 2005;57(6):667-673. doi:10.1016/j.biopsych.2004.12.004.
[29] Kawikova I, Grady BP, Tobiasova Z,Zhang Y, Vojdani A, Katsovich L, et al. Children with Tourette's syndrome may suffer immunoglobulin A dysgammaglobulinemia: preliminary report. Biol Psychiatry.

2010;67(7):679-683. doi:10.1016/j.biopsych.2009.09.034.
[30] Bos-Veneman NG, Olieman R, Tobiasova Z,Hoekstra PJ , Katsovich L, Bothwell AL,et al. Altered immunoglobulin profiles in children with Tourette syndrome. Brain Behav Immun. 2011;25(3):532-538. doi:10.1016/j.bbi.2010.12.003.
[31] Landau YE, Steinberg T, Richmand B, Leckman JF, Apter A. Involvement of immunologic and biochemical mechanisms in the pathogenesis of Tourette's syndrome. J Neural Transm (Vienna). 2012;119(5):621-626. doi:10.1007/s00702-011-0739-x.
[32] Dey P, Kundu A, Sachan R,Park JH,Ahn MY,Yoon K,et al. PKM2 Knockdown Induces Autophagic Cell Death via AKT/mTOR Pathway in Human Prostate Cancer Cells. Cell Physiol Biochem. 2019;52(6):1535-1552. doi:10.33594/000000107.
[33] Hsu MC, Hung WC. Pyruvate kinase M2 fuels multiple aspects of cancer cells: from cellular metabolism, transcriptional regulation to extracellular signaling. Mol Cancer. 2018;17(1):35. Published 2018 Feb 19. doi:10.1186/s12943-018-0791-3.
[34] Bas-Orth C, Tan YW, Lau D, Bading H. Synaptic Activity Drives a Genomic Program That Promotes a Neuronal Warburg Effect. J Biol Chem. 2017;292(13):5183-5194. doi:10.1074/jbc.M116.761106.
[35] Afaf El-Ansary. Activities of key glycolytic enzymes in the plasma of Saudi autistic patients.J Open Access Journal of Clinical Trials,2010,2010(NULL).
[36] Lopes C, Ribeiro M, Duarte AI, Humbert S,Saudou F,Almeida LPet al. IGF-1 intranasal administration rescues Huntington's disease phenotypes in YAC128 mice. Mol Neurobiol. 2014;49(3):1126-1142. doi:10.1007/s12035-013-8585-5.
[37] Naia L, Ferreira IL, Cunha-Oliveira T,Duarte AI,Ribeiro M,Rosenstock TR,et al. Activation of IGF-1 and insulin signaling pathways ameliorate mitochondrial function and energy metabolism in Huntington's Disease human lymphoblasts. Mol Neurobiol. 2015;51(1):331-348. doi:10.1007/s12035-014-8735-4.
[38] Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G. Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling.J Psychiatr Res.2012;46(1):95-104.doi:10.1016/j.jps ychires.2011.09.01.

Figures

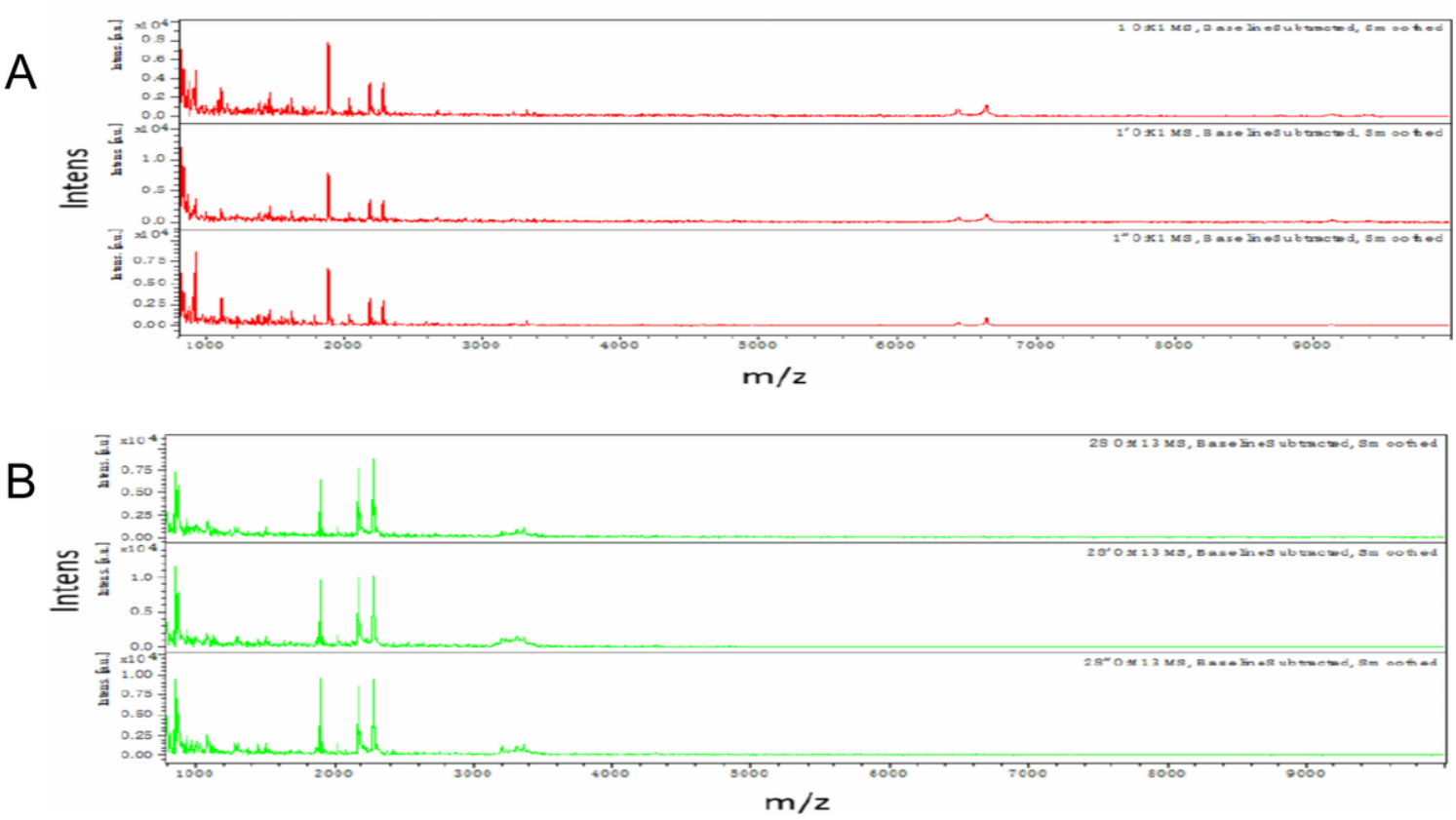

Figure 1

Comparative analysis of serum proteomic profiling between diffffferent groups. A) Representative masss pectra of a healthy control group (green); B) representative mass spectra of an TS group individual (red).

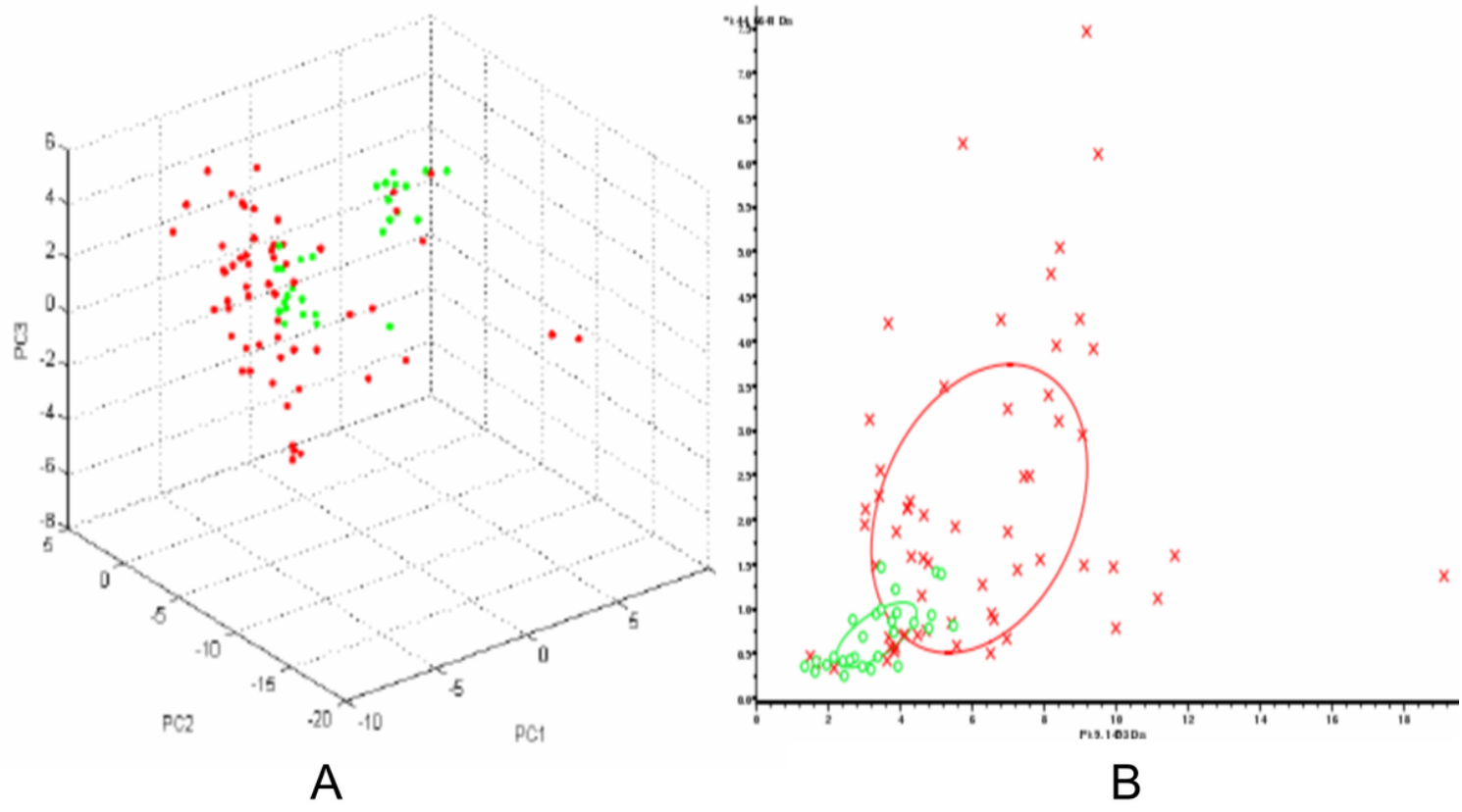

Figure 2

A)Bivariate plot of TS group (red) and healthy control group (green) after subgroup separation in the principal component analysis; B) 3D plot of TS patients (red) and healthy control group (green) after subgroup separation in the principal component analysis.

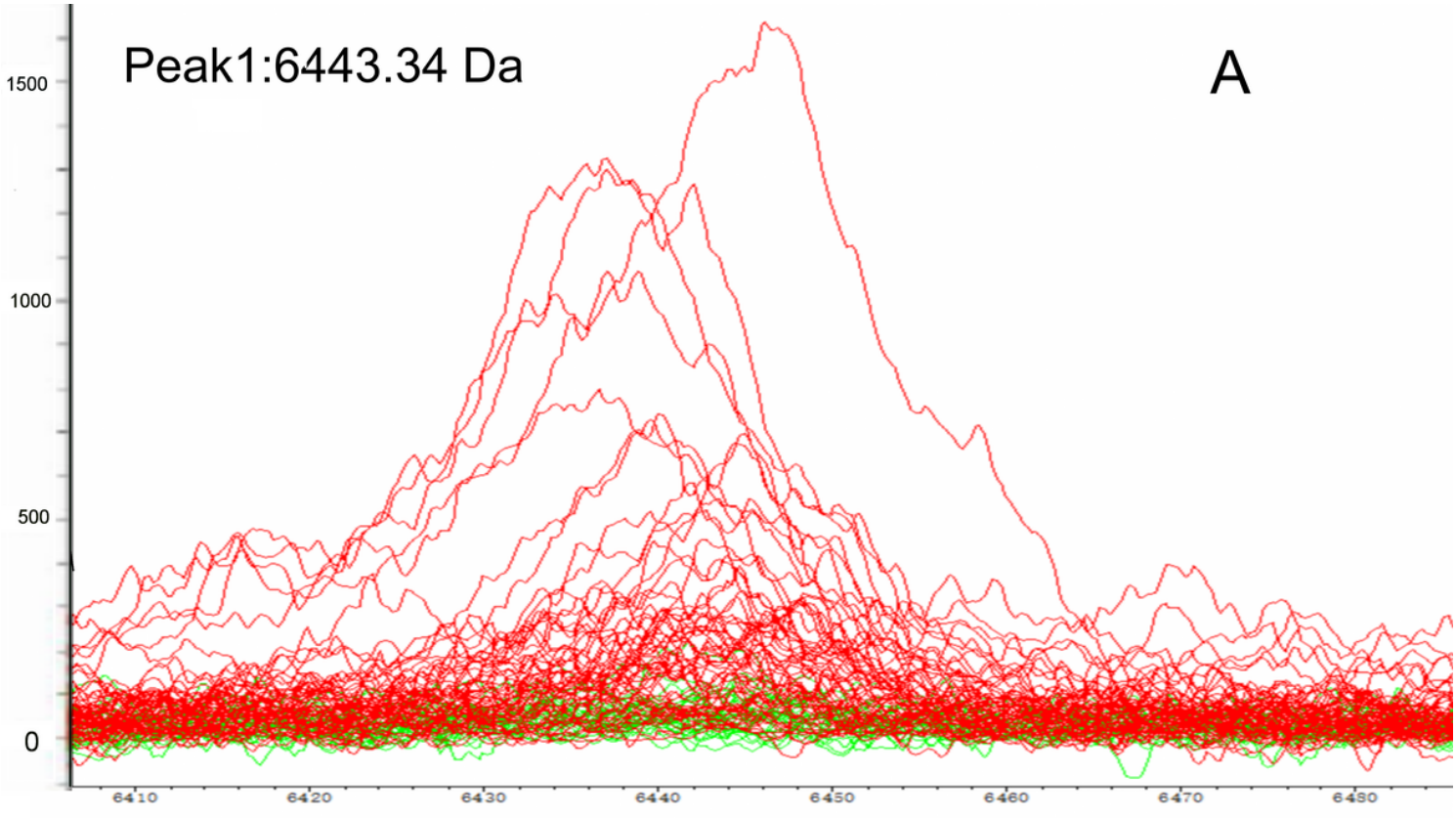

Figure 3

Representative spectra of seven potential serum biomarker peaks in TS group. A,C,E,G,I,K,M) Comparison of the spectra of seven peaks in TS group (red) and health control group (green). B,D,F,H,J,L,N) ROC curves for seven selected peaks with their AUC values.

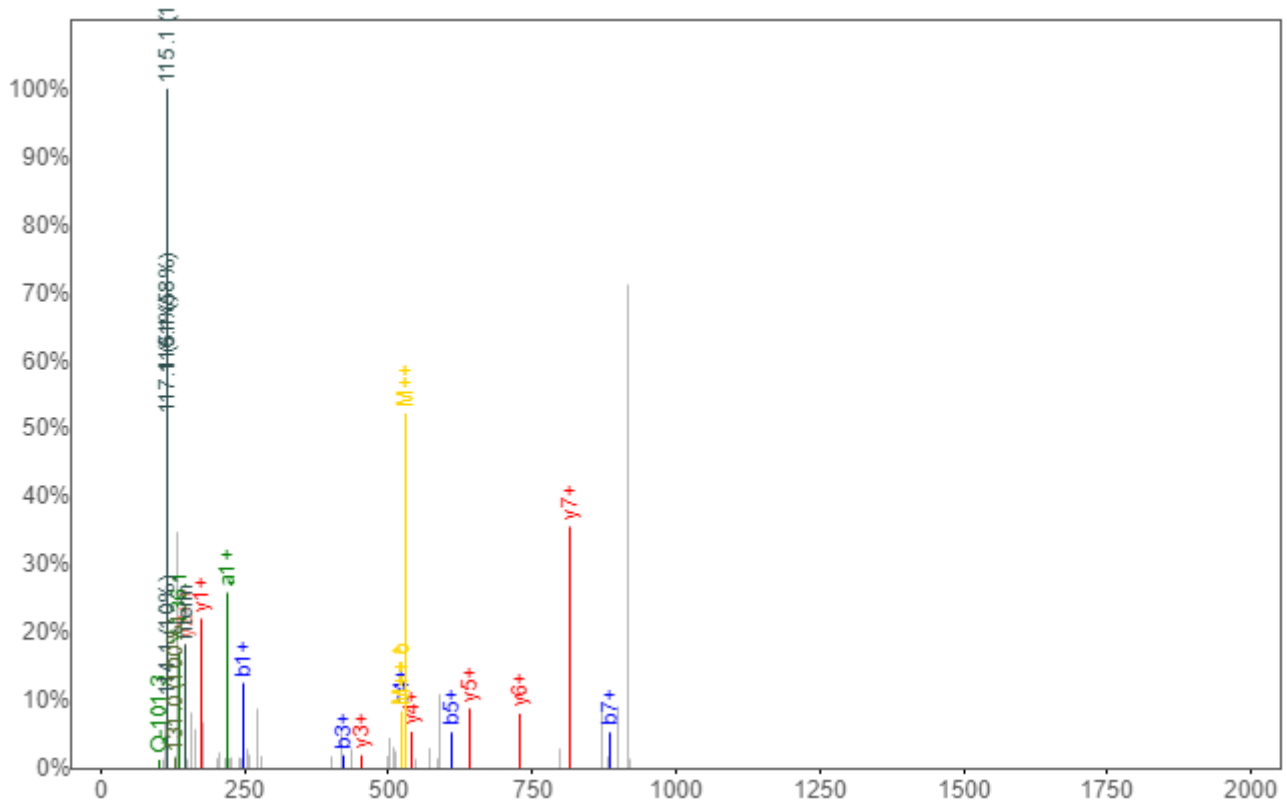

Figure 4

LC-ESI-MS/MS spectrum of peptide 2191.84Da

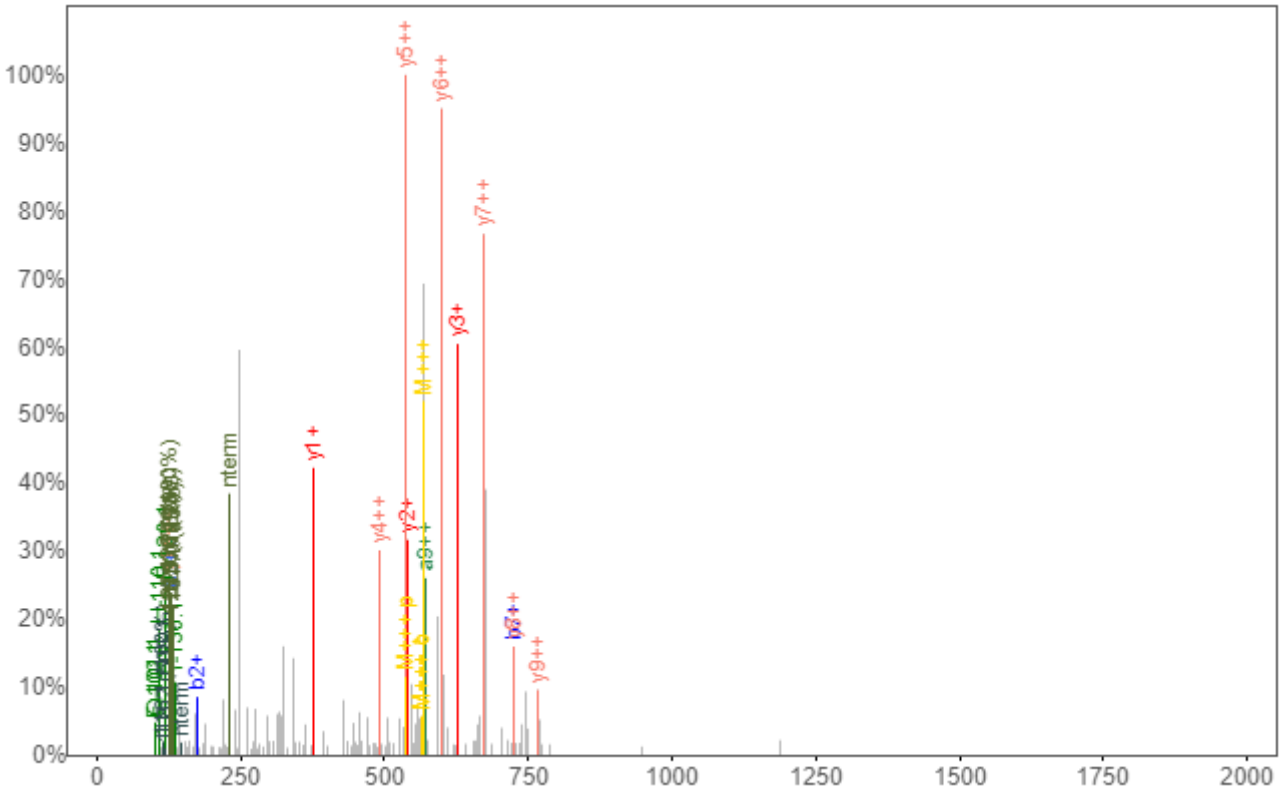

Figure 5

LC-ESI-MS/MS spectrum of peptide 2175.98Da

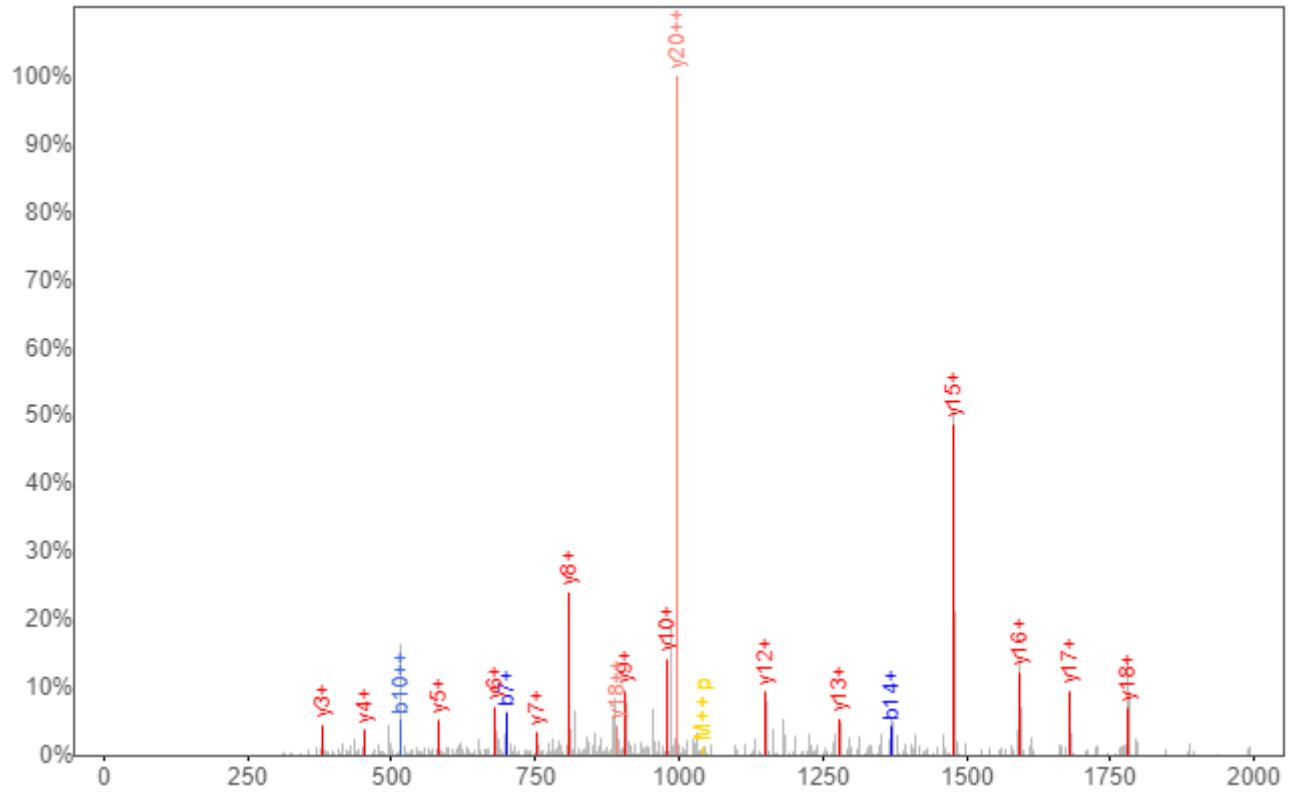

Figure 6

LC-ESI-MS/MS spectrum of peptide 2277.19Da

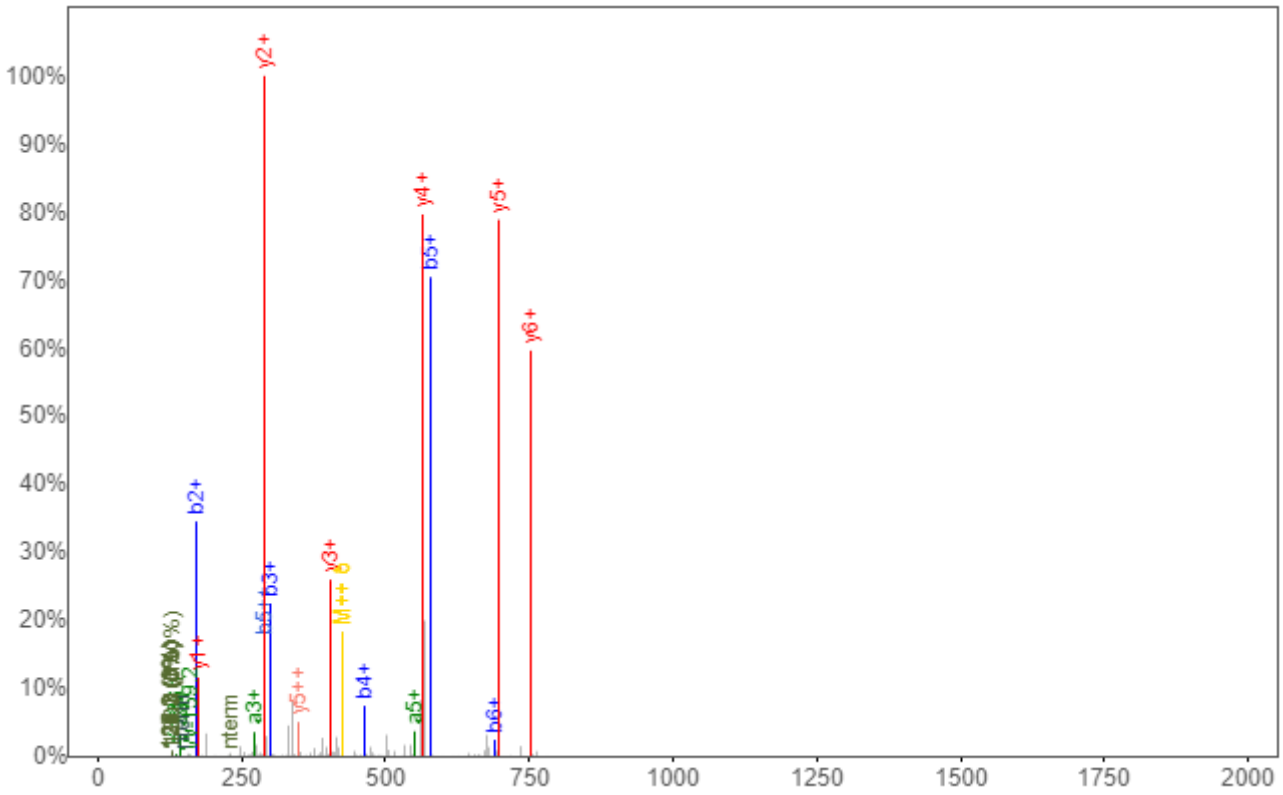

Figure 7

LC-ESI-MS/MS spectrum of peptide 863.13Da

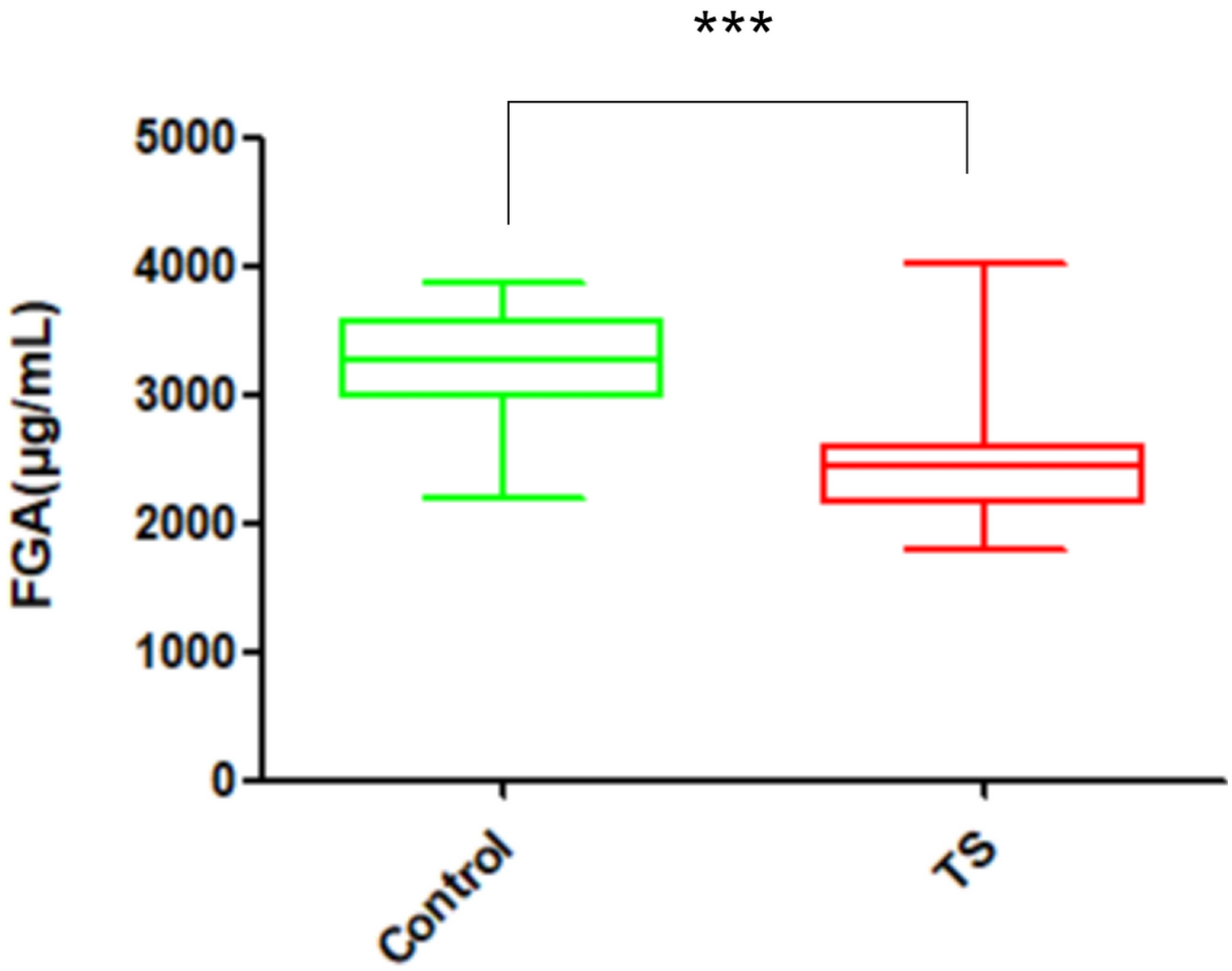

Figure 8

The results of ELISA. ***indicates P<0.0001