Proteomic analysis about the microdeletion of 9q34.3

Jiapeng Zhu [1, 2, a], Yong Dai [2], LangXia Liu [1], Donge Tang [2, b, *]

[1]College of Life Science and Technology, Jinan University, Guangzhou 510632, China

[2]Clinical Medical Research Center, Shenzhen People's Hospital, ShenZhen, 518020, China

[a]350915158@qq.com,[b]donge66@126.com, * Corresponding Author

Abstract

There are many clinical phenotypes occurred in the patients with microdeletion of the 34.3 region on the long arm of chromosome9(9q34.3). Common features between all patients are specific craniofacial features, hypotonia, obesity, microcephaly and mental retardation. However, the molecular mechanism is still unclear. In our study, we found a patient whose karyotype of peripheral blood is normal but cord blood is 9q34.3 microdeletion. Comparing with the normal, we got the differently expressed proteins of the peripheral blood and cord blood in our patient via mass spectrometry. Then, the differentially expressed proteins were analyzed and annotated by means of bioinformatics. These results provided a new sight for illustrating the pathogenesis of 9q34.3 microdeletion.

Keywords

9q34.3 microdeletion, clinical phenotypes, differently expressed protein, analyze.

1. Introduction

Chromosomal variation is the main cause of miscarriage and birth defects. The reason for generation is imbalance of meiosis and mitosis. Chromosome recombination in meiosis and double strand DNA broken could make chromosome terminal unstable and result in terminal delection. 9q34.3 microdeletion is caused by complex chromosomal rearrangements or translocations[1]. It is consist of terminal deletions, interstitial deletions, derivative chromosomes and complex rearrangements. Each of them results in haploinsufficiency of the euchromatin histone methyl transferase 1(EHMT1) gene[2]. All patients with 9q34.3 microdeletion share some important features in common, notably specific craniofacial features, hypotonia, obesity, microcephaly and mental retardation. Others like cardiac defect, genital and limb anomalies were seen in some patients[3].

In our study, we examined the chromosomes of the patient by chromosomes microarray analysis(CMA). Intragenic microdeletion of 1.236 Mb was seen in the 9q34.3 chromosomal region begining at 139784583 and ending at 141020389. According to the DECIPHER database, this region contains 9q34.3 syndrome region. It was reported that the majority of small 9q34.3 deletions (＜1.2 Mb) were paternal, whereas deletions ＞1.2 Mb in size were predominantly maternal in origin[2]. Therefore, the patient whose 9q34.3 deletion of cord blood was more than 1.2 Mb was chosen as our subject.

2. Materials and Methods

2.1 Subject

Peripheral blood (S1) and umbilical cord blood (S2) from the normal gravida were used as the control. Experimental group was the patient whose Peripheral blood(S3) was normal but cord blood(S3) was 9q34.3 microdeletion. This study was approved by the ethics committee of shenzhen people's hospital and obtained informed consent of patients.

2.2 Protein Extraction

Sample was sonicated three times on ice using a high intensity ultrasonic processor in lysis buffer (8 M urea, 1% Protease Inhibitor Cocktail). The remaining debris was removed by centrifugation at

12,000 g at 4 °C for 10 min. Finally, the supernatant was collected and the protein concentration was determined with BCA kit according to the manufacturer's instructions.

2.3 Trypsin Digestion

For digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted by adding 100 mM TEAB to urea concentration less than 2M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion.

2.4 TMT Labeling

After trypsin digestion, peptide was desalted by Strata X C18 SPE column and vacuum-dried. Peptide was reconstituted in 0.5 M TEAB and processed according to the manufacturer's protocol for TMT kit. Briefly, one unit of TMT reagent were thawed and reconstituted in acetonitrile. The peptide mixtures were then incubated for 2 h at room temperature and pooled, desalted and dried by vacuum centrifugation.

2.5 HPLC Fractionation

The tryptic peptides were fractionated into fractions by high pH reverse-phase HPLC using Agilent 300Extend C18 column (5 μm particles, 4.6 mm ID, 250 mm length). Briefly, peptides were first separated with a gradient of 8% to 32% acetonitrile (pH 9.0) over 60 min into 60 fractions. Then, the peptides were combined into 18 fractions and dried by vacuum centrifuging.

2.6 LC-MS/MS Analysis

The tryptic peptides were dissolved in 0.1% formic acid (solvent A), directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 μm i.d.). The gradient was comprised of an increase from 6% to 23% solvent B (0.1% formic acid in 98% acetonitrile) over 26 min, 23% to 35% in 8 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system.

The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive™ Plus coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0s dynamic exclusion. Automatic gain controlwas set at 5E4. Fixed first mass was set as 100 m/z.

2.7 Database Search and Bioinformatics Analysis

The resulting MS/MS data were processed using Maxquant search engine. We used Gene Ontology to analyze the molecular function and protein bioprocesses and KEGG database was used for the enrichment analysis of pathways. SPSS20.0 software was used for statistical processing, and the comparison between groups was conducted by Fisher's exact test. $P<0.05$ was considered statistically significant.

2.8 Western blot analysis

10ug lysate protein from each sample was applied to each lane. After SDS-PAGE, proteins were electroblotted onto PVDF membrane. The membrane was blocked with TBST solution containing 5% skim milk powder for 1h. Then, membrane was incubated at 4℃ overnight with primary antibodies, washing three times and incubated with secondary antibodies for 1h. The proteins were detected by staning membrane with ECL-prime.

3. Results

3.1 Quality control detection

The proteins of sample S1,S2,S3,S4 was identified by mass spectrometry in quantitative proteomics. In order to ensure the accuracy and reliability of the result, we conduct quality control test for mass spectrometry. First, we examined the mass error of all identified peptide. The result showed that mass error is centered on 0 axis and the range was within 10 PPM(Fig.1 A). It indicated that the mass error met the requirements. Moreover, most of the peptides were between 8 and 20 amino acid residues(Fig.1 B). Thus, the sample we preparated also met the standard.

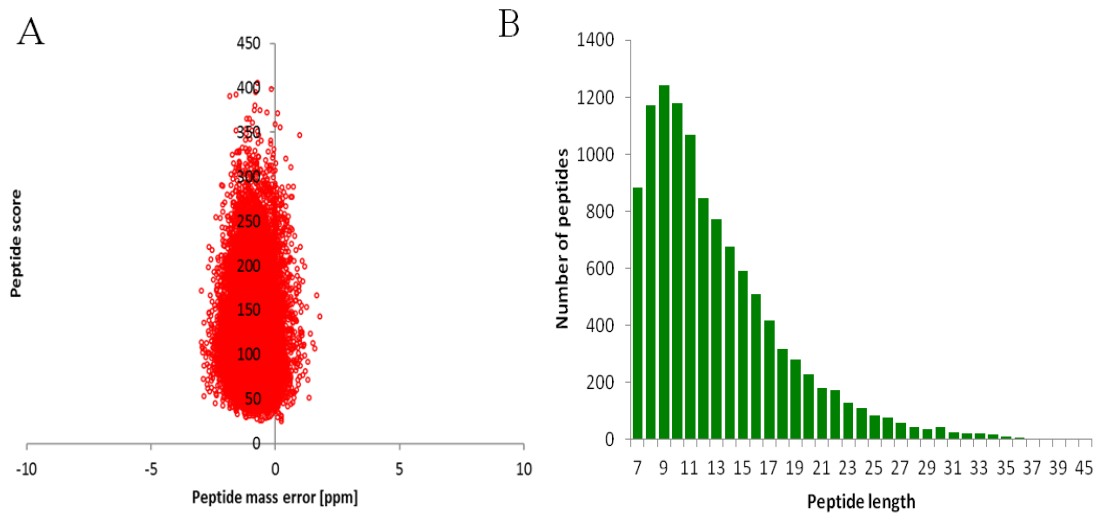


Fig.1 Quality control test results of mass spectrometry data. (A) Distribution of mass error in all peptides. (B) Distribution of the length in all peptides.

3.2 Analysis of Differentialiy expressed proteins

This study identified 1618 proteins, of which 1236 proteins contained quantitative information. The fold change more than 1.5 represented a significant increase, conversely, significant reduction was less than 0.67. $P<0.05$ was considered statistically significant. Therefore, the differently expressed proteins were selected from 1236 proteins. Comparing with the normal, there are 148 up-regulated proteins and 212 down-regulated proteins in patient's peripheral blood. In addition, there are 278 up-regulated proteins and 108 down-regulated protein in patient's cord blood.

3.3 Annotation of molecular function and bioprocesses

The Gene Ontology is a major bioinformatics initiative to unify the representation of gene and gene product attributes across all species. The molecular function and bioprocesses of differently expressed proteins were annotated by Gene Ontology(Fig.2). Difference between the patient and normal was seen in all kinds of molecular function and bioprocesses.

3.4 KEGG Pathway Annotation

KEGG is a network of information connected to known molecules. The KEGG pathway mainly includes: metabolic, genetic information processing, environment information processing,cell process,human disease,drug development and so on. The differently expressed proteins in our study was enriched by KEGG, and the p value of the enriched was converted to negative logarithm (-log10)(Fig.3). From the result, we could know that the abnormality of patient was mainly manifested in the Oocyte meiosis., cell cycle and others. Furthermore, differently expressed proteins of cord blood was related to PI3K/Akt and Notch signaling pathway.

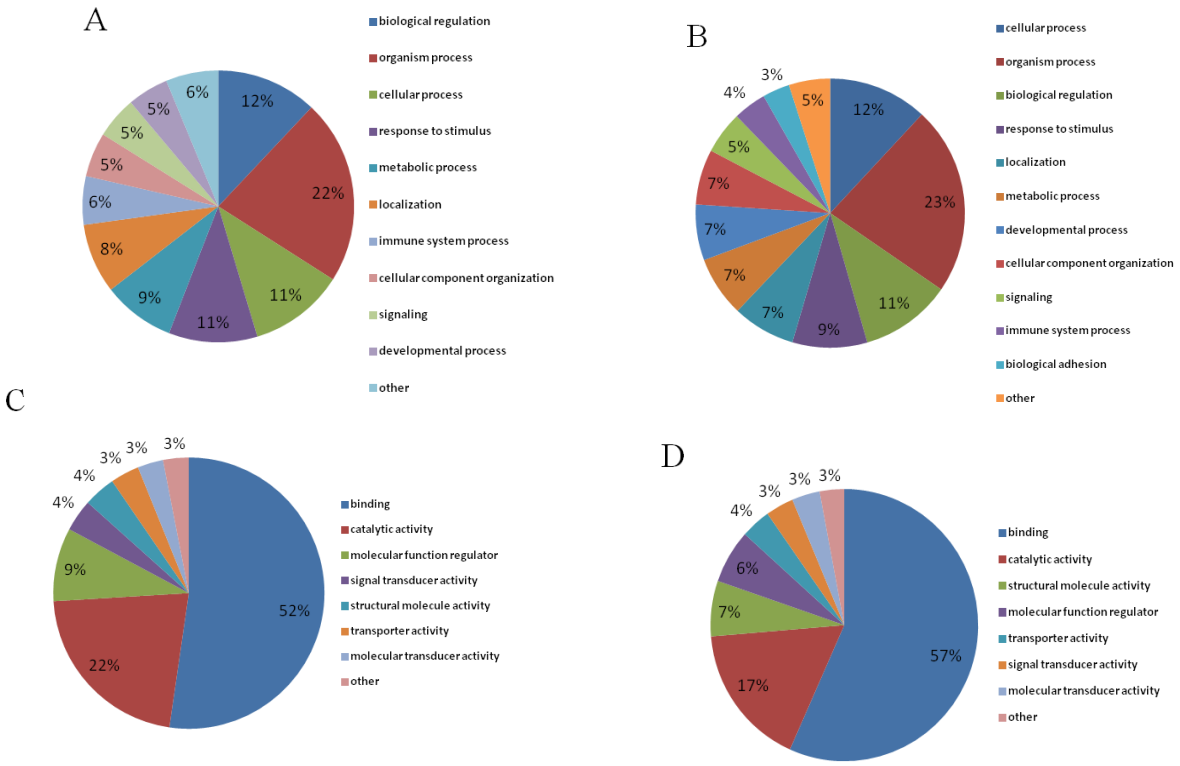
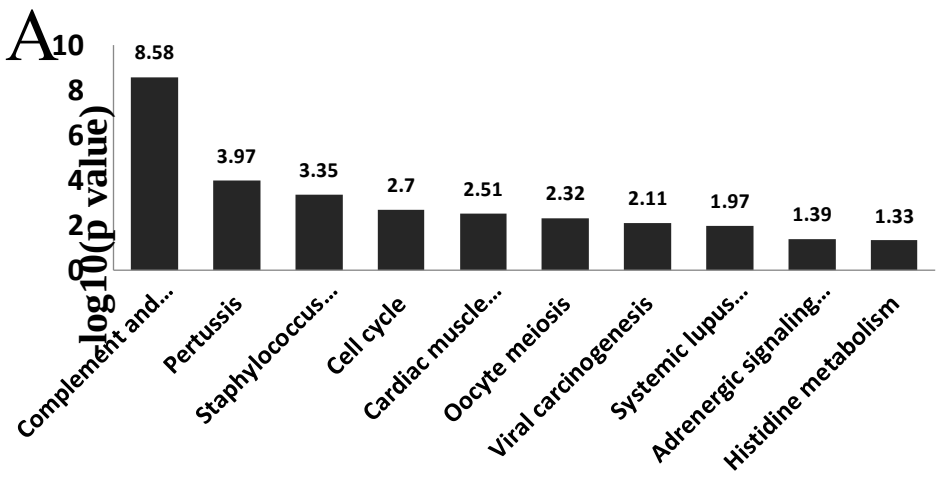


Fig.2 Annotation of molecular function and bioprocesses. Differently expressed proteins of peripheral blood(A) and cord blood(B) were annotated for bioprocesses. Molecular function of differently expressed proteins in peripheral blood(C) and cord blood (D).



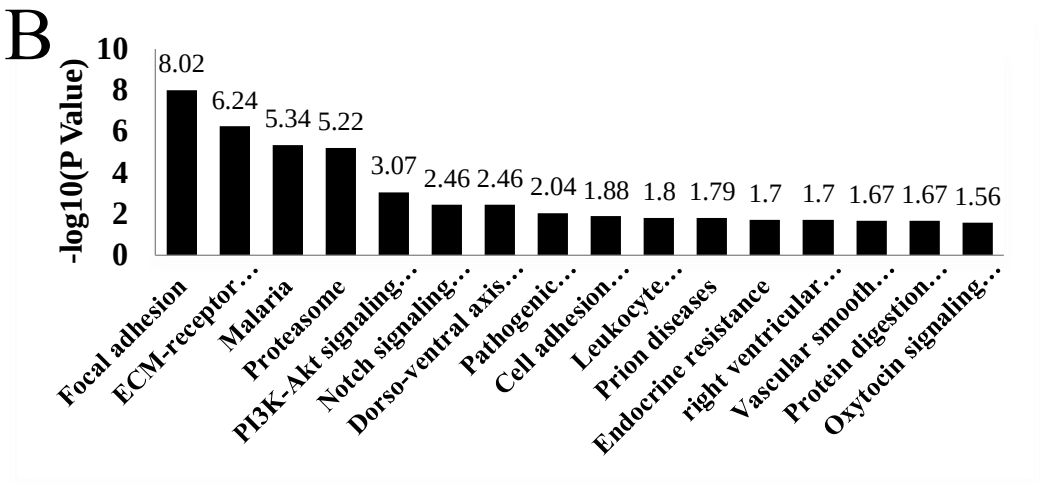


Fig.3 KEGG Pathway Annotation. The differently expressed proteins of peripheral blood(A) and cord blood(B) were annotated by KEGG database.

3.5 Western blot analysis

It has been reported that MAPK and 14-3-3 play an important role in oocyte meiosis[4, 5]. Furthermore, mental retardation and cardiac defect were recongnized in patients with 9q34.3 microdeletion. PI3K/Akt pathway has been confirm to enhance long-term learning and memory in rats[6]. In addition, Notch signaling pathway is closely related to heart development and defect[7, 8]. Therefore, MAPK1, 14-3-3ζ, P-AKT, NOTCH1 were chosen to experiment(Fig.4). Western blot analysis showed that the expression of MAPK1 and 14-3-3ζwere up-regulation in patient's peripheral blood. In patient's cord blood, NOTCH1 was high expression but P-AKT was low. The result of Western blot was consistent with our mass spectrometry data.

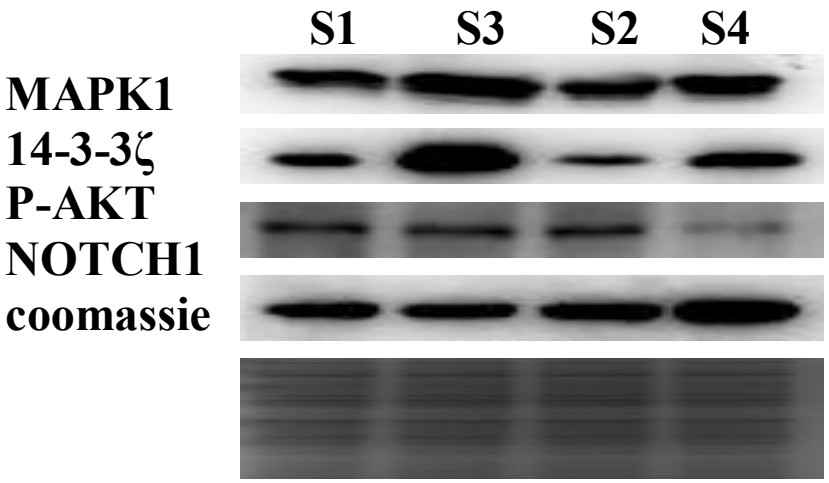


Fig.4 Western blot analysis. The expressed level of MAPK1, 14-3-3ζ, P-AKT, NOTCH1 were detected by Western blot. Coomassie bright blue staining indicates the same amount of sample.

4. Conclusion

In our report, it was the first to analyze the difference between the normal and patient with 9q34.3 microdeletion in proteomics. After preliminary analysis, we believed that the generation of 9q34.3 microdeletion was due to abnormities in oocyte meiosis. Clinical manifestations like mental retardation, cardiac defects, microcephaly were often recongnized in the 9q34.3 microdeletion. Our study indicated that PI_3K-Akt and Notch signaling pathway may be responsible for its' clinical effect. In this study, we have preliminarily elucidated the pathogenesis of 9q34.3 microdeletion. However, further study is needed to explore specific molecular mechanism.

Acknowledgements

The work was supported by Science and Technology Planning Project of Guangdong Province (2017B020209001) and Natural Science Foundation of Guangdong Province(2017A030310629).

References

[1]Willemsen M H, Beunders G, Callaghan M, et al. Familial Kleefstra syndrome due to maternal somatic mosaicism for interstitial 9q34. 3 microdeletions[J]. Clinical genetics, 2011, 80(1): 31-38.

[2]Yatsenko S A, Brundage E K, Roney E K, et al. Molecular mechanisms for subtelomeric rearrangements associated with the 9q34. 3 microdeletion syndrome[J]. Human molecular genetics, 2009, 18(11): 1924-1936.

[3]Samango‐Sprouse C, Lawson P, Sprouse C, et al. Expanding the phenotypic profile of Kleefstra syndrome: A female with low‐average intelligence and childhood apraxia of speech[J]. American Journal of Medical Genetics Part A, 2016, 170(5): 1312-1316.

[4]Wang F, Zhang L, Duan X, et al. RhoA-mediated FMNL1 regulates GM130 for actin assembly and phosphorylates MAPK for spindle formation in mouse oocyte meiosis[J]. Cell Cycle, 2015, 14(17): 2835-2843.

[5]Beaven R, Bastos R N, Spanos C, et al. 14-3-3 regulation of Ncd reveals a new mechanism for targeting proteins to the spindle in oocytes[J]. J Cell Biol, 2017: jcb. 201704120.

[6]Lai Z, Zhang L, Su J, et al. Sevoflurane postconditioning improves long-term learning and memory of neonatal hypoxia-ischemia brain damage rats via the PI3K/Akt-mPTP pathway[J]. Brain research, 2016, 1630: 25-37.

[7]Miazga C M, McLaughlin K A. Coordinating the timing of cardiac precursor development during gastrulation: a new role for Notch signaling[J]. Developmental biology, 2009, 333(2): 285-296.

[8] Sciacca S, Pilato M, Mazzoccoli G, et al. Anti-correlation between longevity gene SirT1 and Notch signaling in ascending aorta biopsies from patients with bicuspid aortic valve disease[J]. Heart and vessels, 2013, 28(2): 268-275.