Identification of novel biomarkers for varicocele using iTRAQ LC-MS/MS technology

Xianfeng Lu[1], Na Li[2], Lufang Li[3], Yongai Wu[4], Xuefeng Lyu[4], Yingli Cao[4], Jianrong Liu[4], Qin Qin[4]

[1]Department of Pediatrics, Shanxi Provincial People's Hospital, Taiyuan, Shanxi 030012, China;
[2]Department of Reproductive Genetics, Heping Hospital Affiliated to Changzhi Medical College, Changzhi, Shanxi 046099, China;
[3]Department of Biochemistry, Basic Medicine College, Shanxi Medical University, Taiyuan, Shanxi 030607, China;
[4]Reproductive Medical Department, Shanxi Provincial People's Hospital, Taiyuan, Shanxi 030012, China.

To the Editor: Varicocele (VC) is a vascular disease and considered as the main cause of male infertility.[1] The incidence of VC in the common male population was 4.4–22.6%, of which the incidence of primary infertility was 35–40%, while the incidence of secondary infertility was as high as 80%.[2] The exact pathophysiological mechanism of male infertility caused by VC and regulative molecules are still unclear. Clear and definite molecular markers of VC disease are helpful for early prevention and timely treatment. This study aimed to screen candidate regulative molecules playing a role in male infertility caused by VC.

This study was approved by the Shanxi Provincial People's Hospital Ethics Committee (No. 2019030), with informed written consent being obtained from all participants. In this study, 12 male Sprague Dawley rats weighing 200–250 g were randomly divided into two groups, a normal group ($n = 3$) and the VC group ($n = 3$), respectively. The rat VC model was referenced from Turner's study.[3] Total proteins extracted from normal and VC rats' testes were tagged with isobaric tags for relative and absolute quantitation (iTRAQ) reagents and analyzed using liquid chromatography-tandem mass spectrometry/mass spectrometry (LC-MS/MS) technology. Differentially expressed proteins were screened between VC and normal rats, and the function of differential proteins was classified based on gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The screened proteins were verified with Western blot in rats' testes. In order to certify the differential proteins, enzyme-linked immunology assay (ELISA) was used to detect the differential proteins in the human seminal plasmas from 30 infertile patients with VC (VC group) and 30 age-matched normal controls (control group) who were recruited from the Department of Reproductive Medicine, Shanxi Provincial People's Hospital from October to December 2019; all participants shared similar demographic characteristics in age, BMI, and endocrine level [Supplementary Table 1, http://links.lww.com/CM9/B733].

Differences between the two groups were compared through independent-sample *t*-test. A *P*-value <0.05 was considered to indicate a statistically significant difference.

A total of 65 differentially expressed proteins were identified compared with the normal group, including 31 up-regulated proteins and 34 down-regulated proteins [Supplementary Figure 1, http://links.lww.com/CM9/B733]. Functions of those proteins were mainly related to the following processes: signal transduction, protein cycle, and so on [Supplementary Figure 2, http://links.lww.com/CM9/B733]. According to literature research, two down-regulated proteins of ATPase and Cu^{2+}-transporting alpha (ATP7A) and calcium and integrin-binding protein 1 (CIB1) were screened due to the correlation with spermatogenesis. Using Western blotting, the expression levels of CIB1 and ATP7A were verified to be lower in VC rat testes than in normal rat testes [Figure 1]. In addition, concentrations of CIB1 in normal and VC semen were 0.17 ± 0.07 ng/mL and 0.10 ± 0.04 ng/mL, and the concentrations of ATP7A in normal and VC semen were 0.58 ± 0.32 ng/mL and 0.20 ± 0.12 ng/mL, respectively. Both proteins were lower in VC semen than in normal semen. There were significant differences between normal and VC semen.

According to the results of proteome analysis, two molecules that affect the spermatogenesis have been selected and verified, and may be used as potential

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002798

Correspondence to: Qin Qin, Reproductive Medical Department, Shanxi Provincial People's Hospital, No. 29, Shuangtasi Street, Taiyuan, Shanxi 030012, China
E-Mail: qinqin@sxmu.edu.cn

Chinese Medical Journal 2024;137(3)
Received: 17-02-2023; **Online:** 25-08-2023 **Edited by:** Yanjie Yin

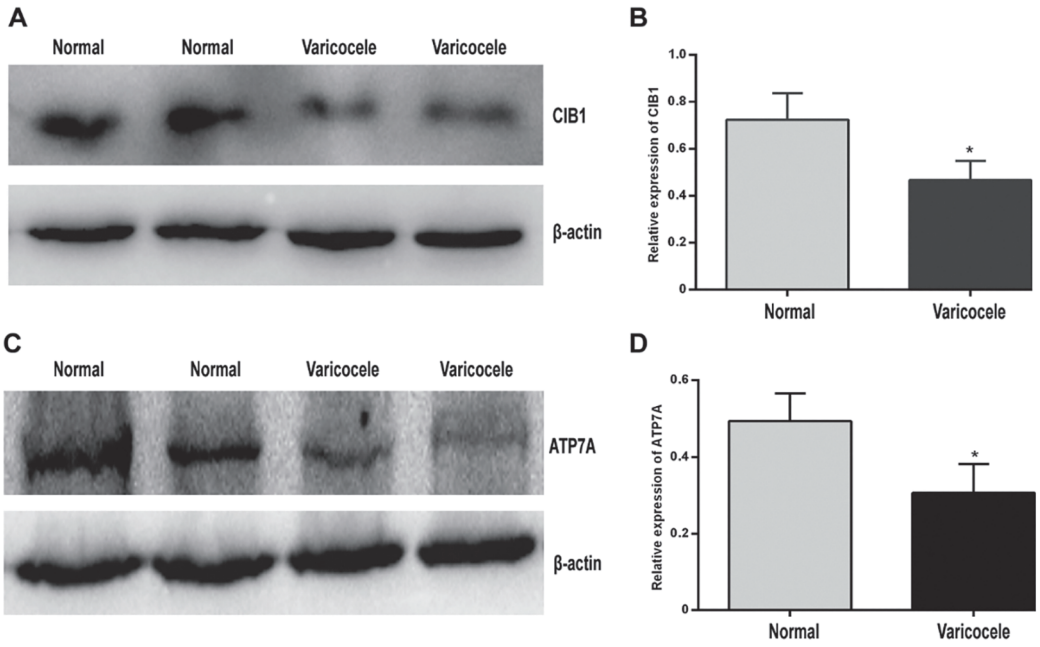

Figure 1: Validation of differentially expressed proteins by Western blotting. β-actin was used as loading control. (A) Western blotting of CIB1 in the varicocele group and normal group. (B) The expression of CIB1 were semi quantified by densitometry. (C) Western blotting of ATP7A in the varicocele group and normal group. (D) The expression of ATP7A were semi quantified by densitometry. $^{*}P$ <0.05 *vs.* normal group.

biomarkers in male infertility with VC, which laid a foundation for further exploration of its role in VC infertility.

Funding

This study was supported by grants from the National Natural Science Foundation of China (Nos. 81401192 and 81973864), Fundamental Research Program of Shanxi Province (No. 20210302123348), and Four "Batches" Innovation Project of Invigorating Medical through Science and Technology of Shanxi Province (No. 2022XM25).

Conflicts of interest

None.

References

1. Sheehan MM, Ramasamy R, Lamb DJ. Molecular mechanisms involved in varicocele-associated infertility. J Assist Reprod Genet 2014;31:521–526. doi: 10.1007/s10815-014-0200-9.
2. Clavijo RI, Carrasquillo R, Ramasamy R. Varicoceles: Prevalence and pathogenesis in adult men. Fertil Steril 2017;108:364–369. doi: 10.1016/j.fertnstert.2017.06.036.
3. Turner TT. The study of varicocele through the use of animal models. Hum Reprod Update 2001;7:78–84. doi: 10.1093/humupd/7.1.78.

How to cite this article: Lu XF, Li N, Li LF, Wu YG, Lyu XF, Cao YL, Liu JR, Qin Q. Identification of novel biomarkers for varicocele using iTRAQ LC-MS/MS technology. Chin Med J 2024;137:371–372. doi: 10.1097/CM9.0000000000002798