Peer Review file

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Review Comments

I think that the team of investigators have great expertise in urine proteomics.
Your article is a great study that identifies biomarkers that can predict abdominal-type HSP. However, I would like to discuss a few more things.

Major

Comment 1- You have identified biomarker for predicting abdominal type HSP in this study. Although the mechanism of HSP itself is not very clear, so far there have been many studies of factors that can predict HSP. Most of these studies were confirmed by comparison with healthy control, and most of those markers are not specific to HSP. It may be elevated from other immune reactions, inflammation, or vasculitis. Although the HSP association was confirmed in the database, the results from this study are expected to be similar. It looks like a little more discussion is needed.
Did the researchers compared the concentrations of the biomarkers in groups with different disease statuses and severity?

Reply 1:

Thanks for your kindly suggestion. We agree with your opinion! Currently, most biomarkers of HSP are not specific and originate from inflammation, immune response or vasculitis. Moreover, the sensitivity and specificity of one single biomarker is usually not enough. Therefore, in this study we establish a biomarker panel to improve the diagnosis performance of HSP and facilitate its future clinical application. As the reviewer suggested, we have added this information in the revised manuscript on page28, lines 469-472.

We have provided the consistent differential urinary proteins between DDA and DIA experiment for comparing the three syndromes (Table S6). As there were a minimum of six urinary proteins (P25774+P09417+Q7Z5L0+P60900+P14550+P09668) is required to form a urinary protein panel, which has the potential for the diagnosis of abdominal type HSP and differential diagnosis of its three subtypes, we therefore listed the fold change between three subtypes in the following as the examples.

Supplementary Table S6. The consistent differential urinary proteins between DDA and DIA experiment for comparing three disease subtypes.

Category	Protein	Protein description	Gene name	Experiment	Average of WH	Average of DP	Ratio of WH/DP	P value
WH vs DP	P14550	Aldo-keto reductase family 1 member A1	AKR1A1	DDA	13.4	4.2	3.19	0.003339
				DIA	206413.4	95967.05	2.15	0.040143

	P60900	Proteasome subunit alpha type-6	PSMA6	DDA	1.2	0.2	6.00	0.013658
				DIA	23628.6	9422.906	2.51	0.017688
Category	Protein	Protein description	Gene name	Experiment	Average of WH	Average of SD	Ratio of WH/SD	P value
WH vs SD	P09668	Pro-cathepsin H	CTSH	DDA	16.2	7.2	2.25	0.008975
				DIA	1527499	692182.9	2.21	0.036196
	P25774	Cathepsin S	CTSS	DDA	5.4	1	5.40	0.000523
				DIA	498177.3	143649.2	3.47	0.034614
Category	Protein	Protein description	Gene name	Experiment	Average of DP	Average of SD	Ratio of DP/SD	P value
DP vs SD	Q7Z5L0	Vitelline membrane outer layer protein 1 homolog	VMO1	DDA	10	17.4	0.57	0.014804
				DIA	2521411	3816847	0.66	0.017548
	P09417	Dihydropteridine reductase	QDPR	DDA	0.6	2.8	0.21	0.030056
				DIA	72660.36	182880.8	0.40	0.002787

Changes in the text: We have modified our text as advised (see Page 28, line 469-472). In addition, we added some data of biomarker abundance among three subtypes of abdominal type HSP (see Table S6).

Comment- 2. Compared to predicting factors of HSP studied in the past, I do not know what advantages and usability are in actual clinical care in terms of price, examination period, and convenience for testing.
Ex) Hong et al. Pediatr Gastroenterol Hepatol Nutr 2015 March 18(1):39-47, Kanik et al. European Journal of Gastroenterology & Hepatology 2015, 27:254-258, Paek et al. BMC Pediatr. 2020 Aug 8;20(1):374
Reply 2:

Thanks for your question. We noticed that these three research articles you mentioned above were using fecal or blood sample for diagnosis. In our study, we use urine sample. Urine is non-invasive than blood, and easy to collect and handling than fecal and blood. In addition, we used the proteomic method in our research to identify a biomarker panel. This biomarker panel can not only be used for HSP diagnosis, but also can be used to differentiate the three subtypes of HSP, which is of great importance for the subsequent personalized treatment of HSP. We believe that this is the capacity that a single protein cannot have in these above research articles. For clinical

application, we can detect multiple proteins in one single experiment through proteomic method, which will be conductive in price and examination period than multiple ELISA examinations in the future.

Comment-3. Of course, the authors also explained the difference according to the three syndromes by traditional Chinese medicine. However, I don't know what it means except for the slight differences explained in the introduction.
Reply 3:

Thanks for your question. These three subtypes of HSP (WH, DP, SD) has different clinical manifestations, and we presented the detailed information on pages 6-7, lines 130-146. And based on these different manifestations, three subtypes of HSP can be distinguished by expert doctors and then patients will be received significantly different drug prescriptions.

In clinical practice, these three symptoms are not easy to distinguish. Thus, from another perspective, phenotyping biomarker is of great significance for precision therapy.

Changes in the text: We have modified our text as advised (see Page 5, line 93-95, pages 6-7, lines 130-146).

Comment-4. Was the urine sample obtained at one point in time with no serial measurements? If it was obtain at one point in time, when was it taken?
Reply 4:

Thanks for your question. All the urine samples in this research were the first midstream morning urine with no serial measurements. In addition, all the urine samples of patients were collected when they first visit the hospital before treatment.
Changes in the text: We have modified our text on page 7, line 148-150 in our manuscript.

Minor
- It would be better to avoid the use of uncommon and unofficial abbreviations. ex)AHSP
Response:
Thanks for your suggestion. We have revised AHSP as abdominal type HSP in our manuscript.