

Letter to the Editor



Perturbations in Amino Acid Metabolism in Children with Kaschin-Beck Disease: A Study of Urinary Target Metabolomics*

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Kaschin-Beck disease (KBD) is an endemic and chronic osteoarthropathy characterized by pathological aspects including chondrocyte degeneration, necrosis, progressive loss of articular cartilage, and secondary degenerative osteoarthritis of epiphyseal cartilage, epiphyseal plate cartilage, and articular cartilage, during puberty^[1]. The main clinical symptoms are limb joint pain, thickening, deformation, limited movement, muscle atrophy, and in case of more severely affected patients, short fingers (toes), short limbs, and even short stature^[1]. In the past, KBD mainly occurred in the population of southeast Siberia, north Korea, and China, whereas nowadays, it is prevalent only in China, with a distribution mainly in narrow areas from the northeast to the southwest. KBD mainly occurs in 6 to 13-year-old children; new cases in adults are rare. Until now, 38,071,000 people are living in the KBD endemic areas and, among a total of 644,994 KBD patients, 16,826 are children under 13 years old^[2]. So far, the causing factors and the pathogenesis of KBD are still to be fully determined despite years of researches. Regarding the identification of biomarkers for KBD, especially the metabolic ones, the studies are limited, and the results are unclear.

This study investigates the association between amino acid (AA) metabolism and KBD in order to discover metabolic biomarkers of this pathology. To this aim, the urinary AAs content in children with KBD and non-KBD was detected by target metabolomics technology.

Boarding school students between 7 and 15 years old, who live in the KBD prevalent regions in Qinghai province (Tangnaihai town in Xinghai county and Gandu town in Hualong county) were selected. Case group and internal control group were generated from these students. As external control

group, a population from Qushian town in Xinghai county, where KBD is not present but the economic level and living habits are similar to the KBD regions, was selected.

Inclusion and exclusion criteria were as follows: hand X-ray imaging was requested for all the children and KBD diagnosis was done according to the KBD diagnostic criteria (WS/T207-2010). Children with positive indications of the disease (metaphyseal wavy or zigzag structure, unevenly thickened bone trabeculae of upper calcification zone and the incomplete, depressed and sclerous of temporary calcification zone. The articular surface of the distal end of phalanx becomes depressed and defective) were included in the case group; children with normal conditions were included in the control group. Children who suffered from other osteoarticular diseases and joint lesions, or who underwent a joint surgery within one year, or followed a therapy for arthritis, were excluded. Children diagnosed with chronic systemic and acute inflammatory diseases were also excluded. The children in control groups were in accordance with cases at the scope of the demographic indexes and other disease indexes. It is worth noting that the daily diet of all children during the school period was unified and supplied by the government (it included cereals, cabbages, potatoes, meat and eggs). So any possible interference from food was eliminated. Finally, 25 KBD children (case group), 41 children with no KBD-linked X-ray changes (internal control group), and 50 healthy children (external control group) were chosen.

Demographic information such as name, gender, age, nationality, height, body weight, past medical history, medication history, and dietary conditions were acquired by a questionnaire.

The study started in July 2014. The morning urine was collected from the subjects and

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centrifuged at 4,000 rpm for 15 min; the supernatant was then collected and frozen at -80 °C.

High performance liquid chromatography-quadrupole ion trap tandem mass spectrometer (HPLC/Q-TRAP-MS/MS) (Beijing Mass Spectrometry Medical Research Co., Ltd) was used for detecting the content of urinary AAs.

Chromatographic separation was carried out by MS Lab-AA-C18 column (150 mm × 4.6 mm, 5 μm). Analytes were eluted from the column with a gradient using water (containing 0.1% formic acid) (A) and acetonitrile (containing 0.1% formic acid) (B) as mobile phase. The column temperature was maintained at 50 °C and the injection volume was 5 μL, with 1 mL/min flow rate. The main parameters of mass spectra are ESI ion source, MRM scanning mode, 5.5 kV IS, and 500 TEM.

SIMCA-P software (version 13.0 Umetrics, Umea) was used for multivariate analysis, and SPSS (version

20.0 IBM, American) software was used to test the normality and the differences in AA content. Chi-square test was used for gender analysis and Kruskal-Wallis test was used for age, height, and body weight analysis. The significance level was set at 0.05.

The general demographic features of the subjects are shown in Table 1. There was no statistical difference in gender among the three groups ($P = 0.7090$), whereas there were statistical differences among the groups in age, height, and body weight ($P < 0.01$).

In total, 50 AAs were detected and quantified; among those, 12 AAs were statistically significant between the three groups, and they included asparagine, taurine, hydroxyproline, β-alanine, carnosine, α-amino adipic acid, γ-aminobutyric acid, aspartic acid, homocitrulline, sarcosine, anserine, and 5-hydroxylysine. The quantitative results are provided in Table 2.

Table 1. The Basic Information of the Population in This Study [M (P_{25} , P_{75})]

Index	External Control Group (n = 50)	Internal Control Group (n = 41)	Case Group (n = 25)	P
Sex (male/female)	25/25	24/17	13/12	0.7090
Age (years)	10.00 (8.00, 10.25)	11.00 (10.00, 12.00)	12.00 (10.00, 13.50)	0.0001 ^{#*}
Height (cm)	129.50 (122.00, 135.00)	136.00 (128.50, 140.00)	143.00 (133.00, 159.50)	0.0001 ^{#*}
Body weight (kg)	26.00 (23.00, 29.00)	30.50 (25.50, 35.00)	34.00 (25.50, 41.00)	0.0001 ^{#*}

Note. Kruskal-Wallis H test, * means statistically significant between case group and external control group, and # means statistical significant between internal control group and external control group.

Table 2. The Quantitative Results of the Difference Amino Acids in this Study [M (P_{25} , P_{75})]

Amino Acids	Case Group (μmol/L)	Internal Control Group (μmol/L)	External Control Group (μmol/L)	P
Asparagine	68.7 (50.6, 125.3) ^a	67.4 (48.6, 98.5) ^a	101.8 (87.4, 130.5) ^b	0.004
Taurine	344.0 (194.2, 6) ^a	321.0 (67.3, 139.8) ^a	735.5 (509.3, 916.8) ^b	0.003
Hydroxyproline	2.4 (1.7, 3.2) ^a	1.9 (1.3, 3.8) ^a	3.5 (2.4, 7.1) ^b	0.004
β-Alanine	1.0 (0.8, 2.8) ^a	1.3 (0.6, 2.5) ^a	3.9 (2.9, 6.5) ^b	0.001
Carnosine	13.9 (4.8, 25.1) ^a	11.3 (7.4, 27.4) ^a	35.7 (21.9, 52.4) ^b	0.001
α-Amino adipic acid	19.4 (15.6, 31.1) ^a	21.6 (12.0, 24.8) ^a	28.0 (23.1, 40.3) ^b	0.002
γ-Aminobutyric acid	1.5 (1.1, 3.0) ^a	1.8 (1.1, 2.7) ^a	2.7 (1.7, 3.1) ^b	0.044
Aspartic acid	3.8 (2.3, 6.3) ^{ab}	3.9 (2.6, 4.9) ^a	5.1 (3.9, 7.2) ^b	0.040
Homo-citrulline	10.9 (8.1, 19.0) ^{ab}	9.4 (5.8, 10.7) ^a	13.0 (9.1, 18.3) ^b	0.031
Sarcosine	1.3 (0.9, 2.2) ^{ab}	0.8 (0.2, 1.9) ^a	2.3 (1.7, 3.7) ^b	0.002
Anserine	2.8 (2.4, 5.4) ^a	1.8 (1.6, 2.4) ^b	3.9 (2.8, 6.7) ^a	0.001
5-Hydroxylysine	7.1 (4.4, 14.1) ^{ab}	5.6 (3.9, 8.9) ^a	9.5 (5.2, 15.7) ^b	0.048

Note. Kruskal-Wallis H test, $\alpha = 0.05$. a and b represented statistically significant among case group, external control group and internal control group, respectively, different letter means difference and one same letter means no-difference.

Specifically, there was a statistical difference in the urinary content of these 12 AAs between external control group and internal control group. Asparagine, taurine, hydroxyproline, β -alanine, carnosine, α -amino adipic acid and γ -aminobutyric acid showed a statistically significant decrease in the case group compared with the external control group. Excluding anserine, the content of the other 11 AAs had no significant differences between case group and internal control group. We also found that the ratios between three branched chain amino acids (BCAAs) and histidine, specifically valine to histidine, leucine to histidine, isoleucine to histidine and total BCAAs to histidine, were statistically significant between case and control group, and all showed a decrease in case group. The results are shown in Table 3.

KBD is a typical endemic disease, and it is doubtless that specific pathogenic factors spread in the KBD areas. After acting on the human body, pathogenic substances will inevitably induce metabolic responses in the body, at cell, tissue and even systemic level, leading to changes in the type and/or concentration of metabolites in the body fluids and to different metabolic disorders. AAs are important metabolites in the metabolism network, participating to the construction of peptides and proteins, and they exert fundamental physiological functions^[3]. Changes in amino acids can have different effects on the body growth and development. Alterations of plasma AAs have been found in inflammatory diseases such as rheumatoid arthritis^[4]; also the establishment and development of osteoarthritis (OA) are associated with inflammation and alterations in the AAs metabolism profiles^[5].

In this study, we measured the urinary AAs content in KBD children using the HPLC/Q-TRAP-MS/MS. We found that 12 AAs were present at lower levels in children from the KBD endemic areas compared with that detected in

children from the non-KBD areas. Compared with the external control group, the level of 7 AAs in the case group was significantly decreased.

Aspartic acid is at the center of the AAs and energy metabolism. Aspartic acid can produce asparagine through transamination; additionally, aspartate can be converted into oxaloacetate and can produce sugars through the gluconeogenic pathway. These processes link the amino acid metabolism with the carbohydrate metabolism, achieving an interchange between amino acid and sugar^[6]. In this study, the trends of aspartic acid and asparagine were consistent, and were much higher in external control group than that in internal control and case group, suggesting that the children in KBD areas might have abnormal AAs metabolism and energy metabolism disorders.

Taurine is a sulfur-containing AA, existing in the body in a free form and is not directly involved in protein synthesis; however, it is still related to the metabolism of AAs. Taurine plays an important role in bone metabolism, which can promote the production of osteoblasts^[7] and inhibits the formation of osteoclasts^[8]. This study showed that the content of taurine in external control group was higher than that in the case group, suggesting that taurine might play a protective role in the development of KBD. These findings provided an insight into the study of biomarkers for KBD and they contribute to future investigations.

A previous study showed that the ratios between BCAAs and histidine were associated with OA^[9]. In this study, the ratios of valine to histidine, leucine to histidine, isoleucine to histidine and the total BCAAs to histidine presented a statistical difference between the case group and the external control group, showing a reduction in the case group. These results highlight the value of the urine as a non-invasive sample, to study bone and joint diseases. However, whether urine can replace blood or joint fluid needs further study.

Table 3. The Ratios of Branched Amino Acids to Histidine [M (P₂₅, P₇₅)]

BCAA Ratio	Case Group	Internal Control Group	External Control Group	P
Valine to histidine	0.048 (0.029, 0.058)	0.056 (0.044, 0.081)	0.062 (0.057, 0.071)	0.007*
Leucine to histidine	0.035 (0.024, 0.045)	0.038 (0.031, 0.053)	0.044 (0.040, 0.054)	0.024*
Isoleucine to histidine	0.170 (0.011, 0.023)	0.019 (0.015, 0.027)	0.024 (0.022, 0.028)	0.003*
Total BCAAs to histidine	0.103 (0.063, 0.122)	0.114 (0.094, 0.160)	0.131 (0.117, 0.152)	0.007*

Note. Kruskal-Wallis *H* test, $\alpha = 0.05$. *Statistical significant between case group and external control group.

In this study, there are some limitations; for example, there were few new cases of KBD with the control of the disease, therefore the statistical analysis of data showed a non-normal distribution. To overcome these limitations, we will increase the sample size and we will further verify our results and conclusion.

In conclusion, AAs metabolic perturbations occur in children affected by KBD; urinary AAs metabolism in children living in KBD areas, showed significant differences compared with healthy children living in non-KBD areas.

All the authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the Journal. None of the authors has any conflicting interests to declare.

This study was conducted in accordance with the Helsinki Declaration II, and was approved by the Institutional Review Boards of Harbin Medical University (HMUIRB 2012001) and the Administration Village Committee. Written informed consents were obtained from the parents or guardians of all children.

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