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Nutrition

Imbalance of dietary nutrients and the associated differentially expressed genes and pathways may play important roles in juvenile Kashin-Beck disease

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ABSTRACT

Background: Kashin-Beck disease (KBD) is a childhood-onset endemic osteoarthropathy in China. Nutrients including trace elements may play active roles in the development of KBD.
Objective: This study aimed to estimate the nutrient intakes of children in endemic areas and to identify the imbalanced nutrients associated differentially expressed genes in the juvenile patients with KBD.
Methods: In this cross-sectional study, a consecutive 3 day 24 h semi-quantitative dietary retrospect questionnaire was conducted to estimate the daily nutrient intakes of children using CDGSS 3.0 software. Gene profile analysis was employed to identify differentially expressed genes in peripheral blood mononuclear cells of children with KBD. GOC, CTD, KEGG, and REACTOME databases were used to establish the relationship between nutrients and nutrients-associated differentially expressed genes and pathways. Statistical analyses were accomplished by SPSS 18.0 software.
Results: Daily Se intakes without supplementation of children were significantly lower in Se-supplemented (Se +) KBD areas (29.3 ~ 29.6 mg/d) and non-endemic area (27.8 ± 7.9 mg/d) compared to non-Se-supplemented (Se-) KBD area (32.9 ± 7.9 mg/d, $c^2 = 20.24$, $P < .01$). Children in Se+ KBD areas were suffering more serious insufficient intake of multiple nutrients, including vitamins-B_2/-C/-E, Ca, Fe, Zn and I. Gene profile analysis combined with bioinformatics technique identified 34 nutrients associated differentially expressed genes and 10 significant pathways which are related to the pathological changes in juvenile KBD.
Conclusions: Imbalance of dietary nutrients and nutrients-associated differentially expressed genes and pathways may play important roles in the development of juvenile KBD.

1. Introduction

Kashin-Beck disease (KBD) is an endemic, childhood-onset, deformative osteoarthropathy with unclear aetiology. KBD has been epidemic in China, Russia and North Korea. China is the prevalent Country with the largest endemic area and the most patients worldwide. There are 378 endemic counties (cities, banners) with more than 104 million residents at risk, among them, including 567.6 thousand patients in the first degree and 12730 juvenile patients under 13 year old [1]. In 2014, the prevalence of KBD was 44.44% in residents from 26 administrative villages of Changdu region in Tibet. In 2015, the prevalence of KBD in children was 25.27% in Tibet [2,3]. These data suggested that KBD in

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China has yet been fully controlled.

Environmental selenium (Se) deficiency has been identified as a main risk factor of KBD, and Se supplementations have been verified effective in preventing and repairing KBD [4]. However, in non-Se-supplemented KBD areas, the incidence has "naturally" declined, and rare new case has been diagnosed in recent years. The reason of this "naturally" regression of KBD remains unclear. Previous studies have found that change in dietary structure may connect with the onset of KBD [5]. Meanwhile, years of investigations have been focused on the relationship between Se deficiency and KBD, thus the potential correlations between other nutrients (including macro and micro elements) and KBD have been ignored.

It has been widely accepted that KBD is a result of environment-gene interaction. Therefore, it is worth exploring the relationship between diet and juvenile KBD from the angle of nutritional genomics. This study integrated the investigation of nutrient intakes of children, microarray analyses of nutrients-related genes and pathways, and bioinformatics technique to provide evidence for offering suggestions to prevent juvenile KBD.

2. Materials and methods

The study protocol was approved by the ethic committee of Xi'an Jiaotong University (Approval no. 2015-070).

2.1. Study design

The research contents and experimental steps of this study are shown in Fig. 1.

2.1.1. Dietary questionnaire investigation

Linyou and Bin Counties in Shaanxi Province were selected as Se-supplemented (Se+) endemic area of KBD. Ningshan and Liquan Counties were selected as non-Se-supplemented (Se−) endemic area and non-endemic area of KBD respectively. Subjects were selected by stratified random sampling method according to matching principle of Se+: Se−: non-endemic = 2: 1: 1. All subjects were local 4–14 years old, Han nationality. Sample size was calculated according to fomula [6] and pre-set response rate. 368 subjects were included for statistical analyses. A consecutive 3 day 24 h semi-quantitative dietary retrospect questionnaire was conducted to collect data of dietary intake of children[7]. 2–4 g occipital hair of children and 20–50 g local produced grain samples were collected. Hydride generation atomic fluorescence spectrometry (HG-AFS) was employed to examine the Se contents of hair and grain samples. EpiData and CDGSS 3.0 were used to input and administrate questionnaire information for evaluating the nutrient intakes of subjects.

Table 1
Demographic characteristics of children, KBD cases and age- and sex-matched healthy controls included for microarray analysis.

Groups	n	KBD			Healthy control		
		Average age (year)	Male	Female	Average age (year)	Male	Female
1	6	12.33	2	1	11.67	2	1
2	6	12.00	2	1	11.67	2	1
3	6	12.33	2	1	12.00	2	1
4	6	11.67	2	1	12.00	2	1
Total	24	12.08	8	4	11.83	8	4

2.1.2. Gene profile analysis and verification experiments

By cluster sampling method, 111 local primary students from Changmu and Bagou Villages in Qinghai Province (non-Se-supplemented endemic area of KBD) were investigated. The subjects were 7–14 years old, Han or Tibetan nationality. According to national diagnostic standard of KBD (WS/T 207-2010) and exclusion of other osteoarthropathy, 19 juvenile patients and 92 healthy subjects were diagnosed. 4 Agilent profile gene chips were designed, 12 juvenile patients (10 patients in early stage and 2 patients in the first degree) and 12 healthy subjects were evenly divided into KBD group and control group. Detailed information of subjects is shown in Table 1. 2.5 mL fasting venous blood from elbow was taken in the morning. Fermentas kit (Thermo Fisher Scientific, America) was used to extract total RNA. Gene profile analysis was then conducted according to the instruction [8].

Deducting the 12 pairs of subjects for gene profile analysis from the 111 primary students, there were 7 juvenile patients and 80 normal children remained, 6 patients and 6 controls with matched age and sex were selected to employ Quantative Real-Time PCR (qRT-PCR). The operating procedures can be learned in previous study [9]. Detailed information of subjects is shown in Table 2. Differentially expressed genes, BIRC3, CYB5A, GPX4, COL1A1, CSGALNACT1 and HAPLN1 were selected as the target genes for qRT-PCR verification since they are involved in KBD pathogenesis related functions, such as apoptosis and extracellular matrix. b-actin was the internal reference. Primers were designed and verified using Oligo 6.0 and Blast-primer software. The primer sequences are shown in Appendix A. Based on the measurement of Ct value, fold change values ($2^{-\triangle\triangle Ct}$) between target gene expressions in KBD group compared with control group were calculated by $\triangle\triangle$Ct relative quantitative method.

According to the former mentioned inclusion and exclusion criteria,

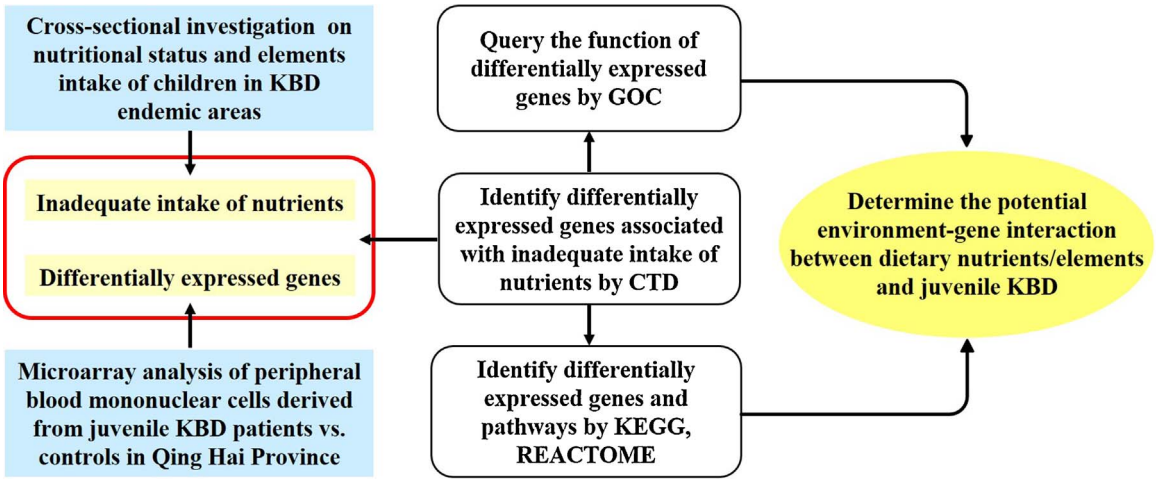

Fig. 1. Study design.