Article

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Investigation of the Potential Mechanism of Maternal Iodine Excess or Iodine Deficiency on Offspring via Untargeted Metabolomics

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Background: Iodine plays a key role in various functions of the central nervous system, and both iodine deficiency and excess are harmful to health. This study aimed to explore the potential metabolites and pathways involved in the effects of excess maternal iodine or iodine deficiency on offspring.

Methods: All female rats were randomly grouped into severe iodine deficiency (SID), mild iodine deficiency (MID), normal iodine (NI), mild iodine excess (MIE), and severe iodine excess (SIE), and the corresponding model was constructed based on the water iodine intervention. After successful conception, the same feeding conditions were maintained for the pregnant rats. On the 21st day of pregnancy, serum samples of the offspring were collected for liquid chromatography-tandem mass spectrometry and bioinformatics analyses.

Results: With the increase in the iodine intervention dose, the median urinary iodine of rats, as well as their free thyroxine levels and positive rate of thyroglobulin antibody, were also elevated ($p < 0.05$), suggesting that the model was successfully constructed. By analyzing the serum metabolites of offspring, 173, 186, 66, and 92 differential metabolites, such as L-glutamic acid and deoxycytidine, were found to be significantly altered in the severe iodine deficiency SID vs. the normal iodine, the mild iodine deficiency vs. the normal iodine, the mild iodine excess vs. the normal iodine, and the severe iodine excess vs. the normal iodine groups, respectively. Pathway enrichment analysis revealed that ATP-binding cassette (ABC) transporters were the most commonly enriched metabolic pathways.

Conclusion: These findings suggest that maternal iodine deficiency or excess may affect the development of offspring by regulating metabolites such as L-glutamic acid and deoxycytidine via the ABC transporter pathway.

Keywords: iodine excess; iodine deficiency; untargeted metabolomics; liquid chromatography tandem mass spectrometry analysis; bioinformatics analysis

Introduction

Iodine is an essential nutrient for thyroid hormone synthesis, and regulates various physiological functions in human growth and development [1,2]. Particularly, iodine plays a key role in the differentiation, development, and formation of various functions of the central nervous system [3]. Both iodine deficiency and excess are harmful to human health. In children, iodine is one of the most common causes of preventable mental retardation [4], which may lead to decreased physical development, poor school performance, and reduced resistance to infection [5]. Usually, adequate iodine intake during infancy is essential to ensure the optimal storage of thyroid hormones and prevent impaired neurodevelopment [6]. A previous report showed that the fetus receives all its thyroid hormones from the mother's thyroid for brain development for up to 12 weeks [7]. More importantly, it has been widely reported that the thyroid function of the mother during pregnancy is closely related to that of the fetus and that the thyroid function of the mother directly affects the growth and development of the next generation [8,9]. Studies have shown that in the serum of pregnant rats with excess iodine, low levels of free triiodothyronine (FT3), high levels of free thyroxine (FT4), thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb) are detected [10]. Meanwhile, increased maternal FT4 was associated with an increased psychomotor developmental index in offspring [11]. In addition, iodine deficiency can lead to elevated maternal thyroid-stimulating hormone (TSH) levels, which are associated with increased risks of premature birth and neonatal respiratory distress syndrome [12]. Therefore, appropriate iodine intake during pregnancy is essential for fetal development.

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As a promising clinical tool, metabolomics can be used to evaluate human health and disease status by detecting and analyzing metabolites in blood, urine, and stool samples. Metabolites can also be used as biomarkers of many diseases [13–15]. Metabolomic technologies, especially untargeted metabolomic approaches, have been widely used to explore novel mechanisms of different diseases [16–19]. In this study, to explore the key metabolites and metabolic pathways involved in offspring development affected by maternal iodine excess or deficiency, models of maternal iodine excess or deficiency were constructed, and progressive changes in maternal thyroid hormone levels were observed. Key metabolites and metabolic pathways were screened based on untargeted metabolomic analysis. Our findings reveal the potential mechanism of maternal iodine excess or iodine deficiency in offspring, which will provide insights into the pathogenesis of some iodine-deficiency diseases.

Materials and Methods

Animals and Grouping

A total of 150 female Wistar rats and 30 male Wistar rats weighing at 157 ± 18 g and aging at 2 weeks were obtained from Xinjiang Experimental Animal Research Center (License number: [SCXK (Xin) 2016-0001]). All animals were kept in the specific pathogen-free laboratory of Xinjiang Experimental Animal Research Center [SYXK (Xin) 2015-0001] at a constant temperature of 23~24 °C and 45%~60% humidity, maintaining a 12-h light/dark cycle. This animal experiment was approved by the Xinjiang Uygur Atonomous Region Center for Disease Control and Prevention Ethical Review Committee (No: 202002-001) and all procedures followed the Guide for the Care and Use of Laboratory Animals mandated by the National Institutes of Health. During the course of the experiment, female Wistar rats were fed a low-iodine animal feed (number: D18503, Beijing keao xieli Feed Co., Ltd., China), with an average daily feed of 25 g and an iodine content of 60 ng/g. Low-iodine animal feed was prepared according to the Nutrition Components of Compound Feed for Experimental Animals guidelines (GB14924.3-2010).

After a one-week acclimatization period, female rats were randomly assigned into severe iodine deficiency (SID), mild iodine deficiency (MID), normal iodine (NI), mild iodine excess (MIE), and severe iodine excess (SIE). Iodine nutrition in female rats was achieved by administering deionized water containing potassium iodide. Iodine concentrations in SIE, MIE, NI, MID, and SID groups were 11,617, 2283, 183, 50 and 0 μg/L, respectively. Based on an average daily water intake of 30 mL and average daily feed of 25 g, the iodine intake in the SIE, MIE, NI, MID, and SID groups was estimated to be 350, 70, 7.0, 3.0 and 1.5 μg/day, respectively [20]. After three months of water-iodine intervention, urine was collected in a metabolic cage for 24 h to determine urinary iodine. Successful modeling was defined as a sequential increase in the urinary iodine value in each group with a statistically significant difference. To anesthetize the rats, a 1% pentobarbital sodium (57-33-0, Sigma, St. Louis, MO, USA) solution was administered intraperitoneally at a dose of 50 mg/kg, and the thyroid hormone levels of rats in different groups were detected by separating serum from 3 mL of abdominal aortic blood. Five indices, free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), thyroglobulin antibody (TgAb), and thyroid peroxidase antibody (TPOAb), were measured using an electrochemiluminescence immunoassay (E411, Roche, Mannheim, Germany). The male rats were fed a standard iodine-containing diet and water.

Sample Collection and Preparation

After the model was successfully established, female rats were mated with normal male rats in a ratio of 3:1. Copulation of the rats was determined on the day the vaginal plug appeared. Each group comprised at least 15 pregnant rats. Pregnant rats were fed under the same conditions from the nongestational period to the 21st day after pregnancy (PN21). On PN21, serum samples from the offspring (20 females and 20 males) were collected, followed by metabolomic analysis.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis

After sample pretreatment, the target compounds were isolated by the ACQUITY UPLC BEH column (2.1 mm × 100 mm, 1.7 μm, Waters, Milford, MA, USA) at 40 °C with a flow rate of 0.3 mL/min, followed by the gradient elution of metabolites. Each sample with a loading volume of 5 μL was separated by ultra-high-pressure liquid chromatography and analyzed using a Thermo Q-Exactive mass spectrometer (14017288, Thermo Fisher Scientific, Cleveland, OH, USA), with simultaneous detection by electron spray ionization "+" and "–" ion modes.

Data Processing and Bioinformatic Analyses

Raw data processing, including peak extraction, alignment, correction, and standardization, was performed using Compound Discoverer 3.0 (Thermo Fisher Scientific, Cleveland, OH, USA). Biocyc (http://BioCyc.org/), The Human Metabolome Database (HMDB, http://www.hmdb.ca), metlin (https://metlin.scripps.edu), the Human Fecal Metabolome Database (HFMDB, https://www.fecalmetabolome.ca/), and Lipidmaps (http://www.lipidmaps.org) were used to identify and match metabolites. After pattern recognition using SIMCA-P (version 14.1; Umetrics, Umea, Sweden) and Pareto-scaling processing of the data, multivariate statistical analyses including principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were performed.

Table 1. Comparison of urinary iodine levels in rats with different doses of water iodine (μg/L) [21].

Groups	Samples (N)	Median, interquartile interval (M, *p*25∼*p*75)	Statistic	*p*
SID	30	3.540 (0.116∼8.080)	Z = –5.416	0.000*
MID	30	51.410 (40.084∼69.316)	Z = –5.410	0.000*
NI	30	286.801 (250.644∼337.941)		
MIE	30	644.192 (595.813∼707.421)	Z = –5.361	0.000*
SIE	30	2368.701 (2060.541∼3264.677)	Z = –5.410	0.000*

**p* indicates statistical significance. SID, severe iodine deficiency; MID, mild iodine deficiency; NI, normal iodine; MIE, mild iodine excess; SIE, severe iodine excess.

With the variable importance in the projection (VIP) value in OPLS-DA >1 and p value < 0.05, differential metabolites were screened. A cluster heatmap of the identified metabolites was constructed using the R.Pheatmap package (version:0.6.1; R Core Team, Vienna, Austria). The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.kegg.jp) was used to investigate the enriched pathways of the differential metabolites. Significance thresholds were set at a count ≥2 and $p < 0.05$.

Statistical Analysis

SPSS software (version 22.0; SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis. One-way Analysis of Variance (ANOVA) and non-parametric Kruskal-Wallis rank sum test were used for overall comparison between measurement data groups, and least significant difference (LSD) and *t*-test were used for further multiple comparisons. A $p < 0.05$ was considered statistically significant.

Results

Urinary Iodine and Thyroid Hormones Levels of Rats in Different Groups

The urine iodine content of the five groups of rats was measured after three months of water iodine intervention. As shown in Table 1 (Ref. [21]), the median urinary iodine level of rats in the five groups increased with an increase in the iodine intervention dose in the drinking water. The non-parametric Kruskal-Wallis rank sum test showed a statistically significant difference in urinary iodine content among the five groups ($\chi^2 = 94.791$, $p < 0.001$). After multiple tests, there was a statistically significant difference in urinary iodine content between the groups and the NI group (Z = –5.416, –5.416, –5.361, –5.41, $p < 0.001$), suggesting that the model was successfully constructed. Thyroid hormone levels were also measured, and the results suggested significant differences in FT4, FT3, and TgAb positive rate among the five groups ($p < 0.05$). The level of FT4 and the positive rate of TgAb were elevated with an increase in the iodine intervention dose (Table 2, Ref. [21]).

Quality Control

Total ion chromatograms (TIC) of the quality control samples were compared using spectral overlap. As shown in Fig. 1, the response intensity and retention time of each chromatographic peak overlapped, illustrating that the variation caused by instrumental errors was small throughout the experiment.

Multivariate Statistical Analyses of the Metabolic Profiles and Differential Metabolites Screening

PCA was conducted to classify the samples from patients with iodine deficiency/excess and normal controls. As illustrated in Fig. 2, the PCA results show a clear separation tendency for the positive and negative modes. Additionally, the OPLS-DA results for the SID group vs. NI group, MID group vs. NI group, MIE group vs. NI group, and SIE group vs. NI group showed that the different groups exhibited a clear separation trend (Fig. 3), with both R2Y and Q2Y over 0.05 (Fig. 4), indicating that the model for grouping was stable and reliable. Furthermore, a total of 854, 745, 594, and 603 metabolic peaks with VIP values larger than 1.0, in the SID group vs. NI group, MID group vs. NI group, MIE group vs. NI group, and SIE group vs. NI group, respectively, were considered for subsequent analysis (**Supplementary Fig. 1**). Finally, using the univariate *t*-test (predicted by the p value < 0.05, FC >2.0), VIP >1, 173, 186, 66, and 92 differential metabolites were found to be significantly altered in the SID group vs. NI group, MID group vs. NI group, MIE group vs. NI group, and SIE group vs. NI group, respectively. Among these, L-glutamic acid may be associated with maternal iodine deficiency, whereas deoxycytidine production may be influenced by excess maternal iodine. In addition, the levels of n-propyl GalIate, 4-(4-Nitrobenzyl)pyridine, and p-nitrophenyl-O-ethyl ethylphosphonate were found to be significantly higher in iodine-deficient rats than in iodine-excess rats, suggesting that the levels of these metabolites in offspring were negatively correlated with maternal iodine intake. Clustering heatmaps are shown in **Supplementary Figs. 1,2,3,4**.

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Table 2. Comparison of thyroid hormone levels in rats with different doses of water iodine [21].

Groups	Samples	TSH	FT4	FT3	TgAb	TPOAb
	(N)	Median, interquartile interval (M, $p25 \sim p75$) (mIU/L)	X ± s (pmol/L)	X ± s (pmol/L)	Positive rate (%)	Positive rate (%)
SID	16	0.818 (0.458~2.260)	8.683 ± 2.088	7.032 ± 1.767	0.00 (0)	0.00 (0)
MID	15	0.785 (0.460~1.858)	17.051 ± 3.933	5.318 ± 0.889	6.67 (1)	6.67 (1)
NI	15	0.589 (0.459~0.662)	16.955 ± 3.760	5.537 ± 0.463	6.67 (1)	6.67 (1)
MIE	15	0.542 (0.521~0.681)	19.517 ± 4.833	5.624 ± 0.585	6.67 (1)	0.00 (0)
SIE	14	0.617 (0.499~0.703)	19.277 ± 5.551	5.537 ± 0.796	42.86 (6)	14.29 (2)
Statistic		$\chi^2 = 3.329$	F = 7.389	F = 4.299	$\chi^2 = 4.474$	$\chi^2 = 0.233$
p		0.504	0.000*	0.005*	0.034*	0.629

*p indicates statistical significance. TSH, thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody.

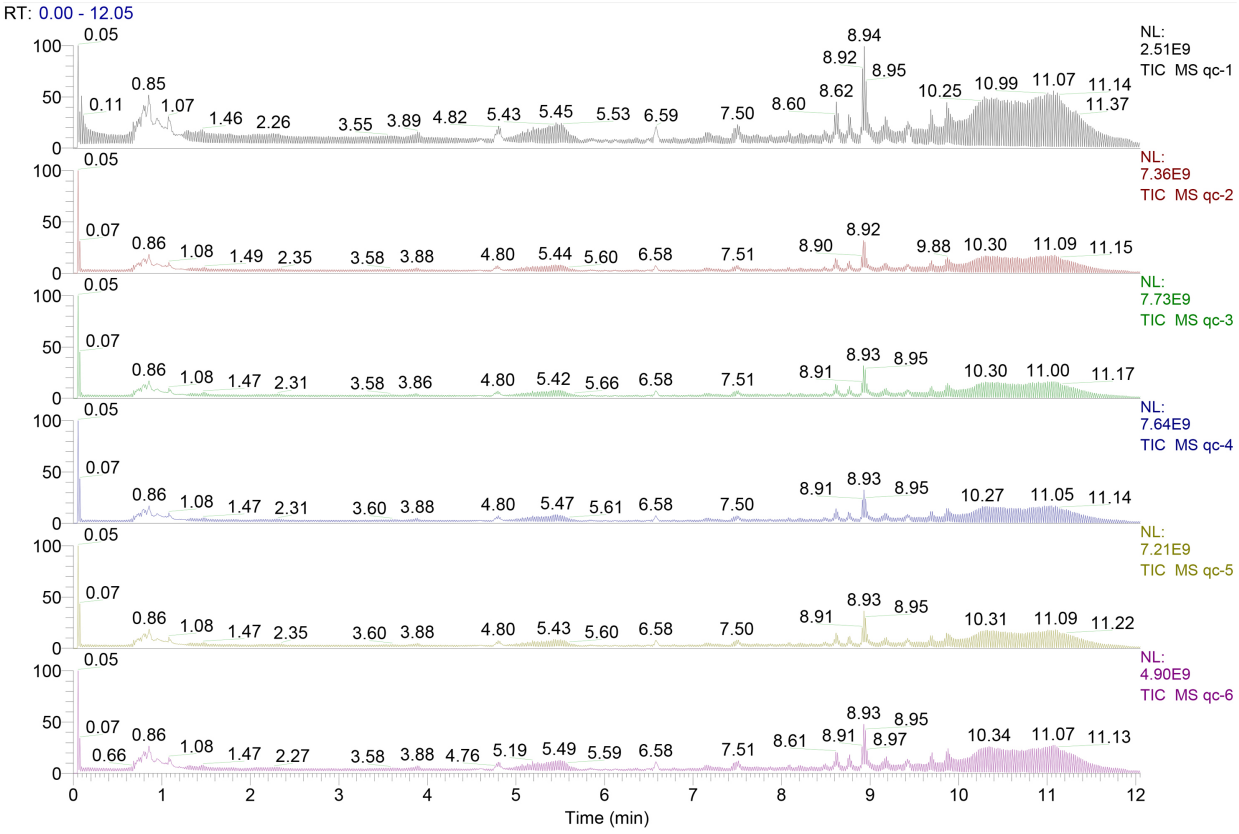

Fig. 1. The total ion chromatograms (TIC) of quality control sample.

Pathway Enrichment Analysis

To explore the possible involvement of differential metabolites in these pathways, enrichment analysis was performed (**Supplementary Fig. 2**). The results showed 131 pathways, such as taurine and hypotaurine metabolism, serotonergic synapse, and ATP-binding cassette (ABC) transporters, were identified based on the differential metabolites in SID group vs. NI group; 129 pathways, such as beta-Alanine metabolism, synaptic vesicle cycle, and ABC transporters, were identified in MID group vs. NI group; 92 pathways, such as ABC transporters, taurine and hypotaurine metabolism, and neuroactive ligand-receptor interaction, were identified in MIE group vs. NI group; as well as 121 pathways, such as central carbon metabolism in cancer, alanine, aspartate and glutamate metabolism, and ABC transporters, were identified in SIE group vs. NI group, respectively. Among these, the ABC transporter pathway is the most significant. The top 10 significantly enriched pathways are shown in Fig. 5. Among them, differential metabolites in the SID vs. NI group and MID vs. NI group were mainly enriched in bile secretion pathways, whereas ABC transporters and central carbon metabolism in cancer pathways were most significant in the MIE vs. NI group and SIE vs. NI group, respectively.

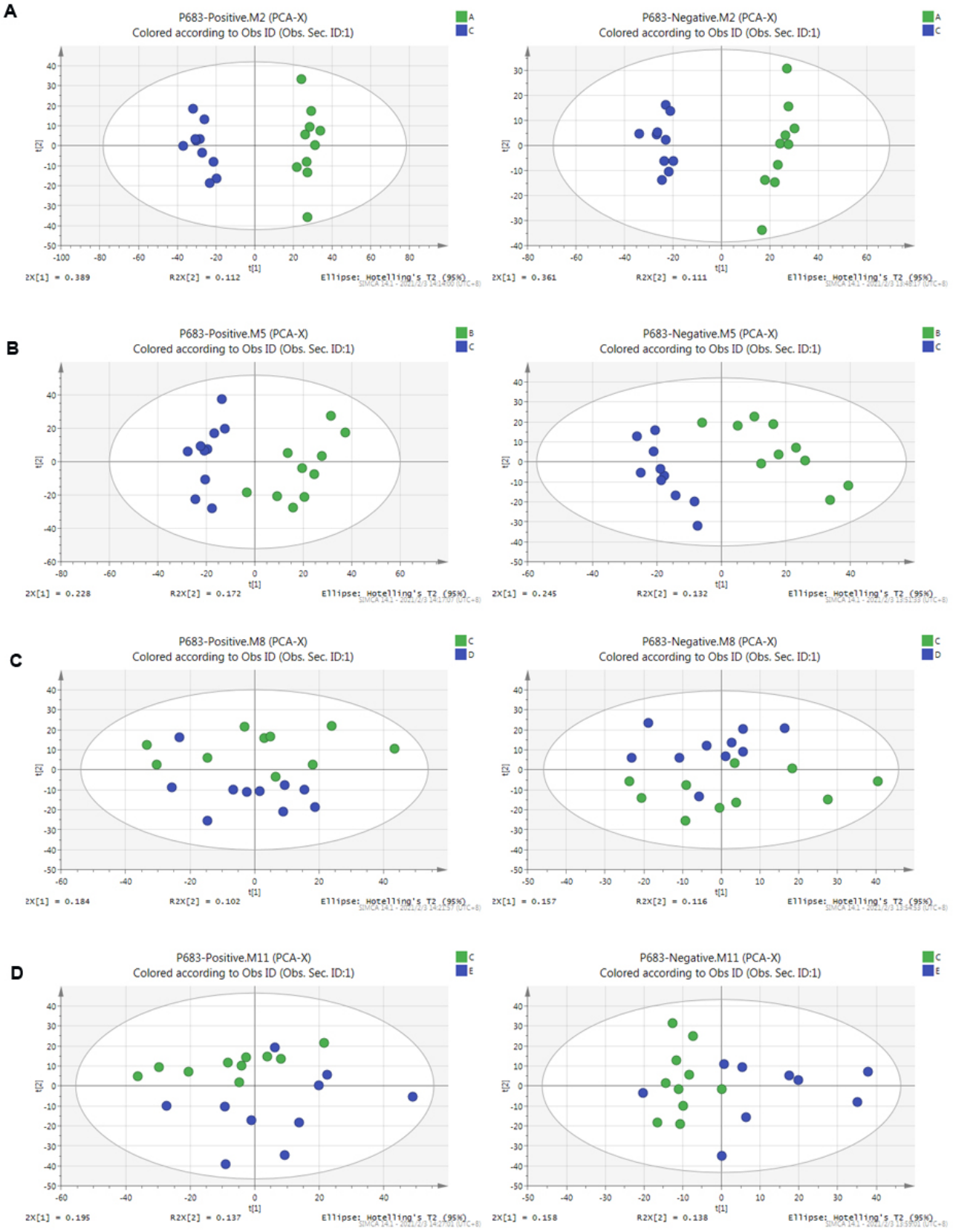

Fig. 2. The scatter plots of principal component analysis (PCA) based on the metabolic data in + and – ion modes. (A) SID group vs. NI group; (B) MID group vs. NI group; (C) MIE group vs. NI group; (D) SIE group vs. NI group. A: SID group; B: MID group; C: NI; D: MIE group and E: SIE group.

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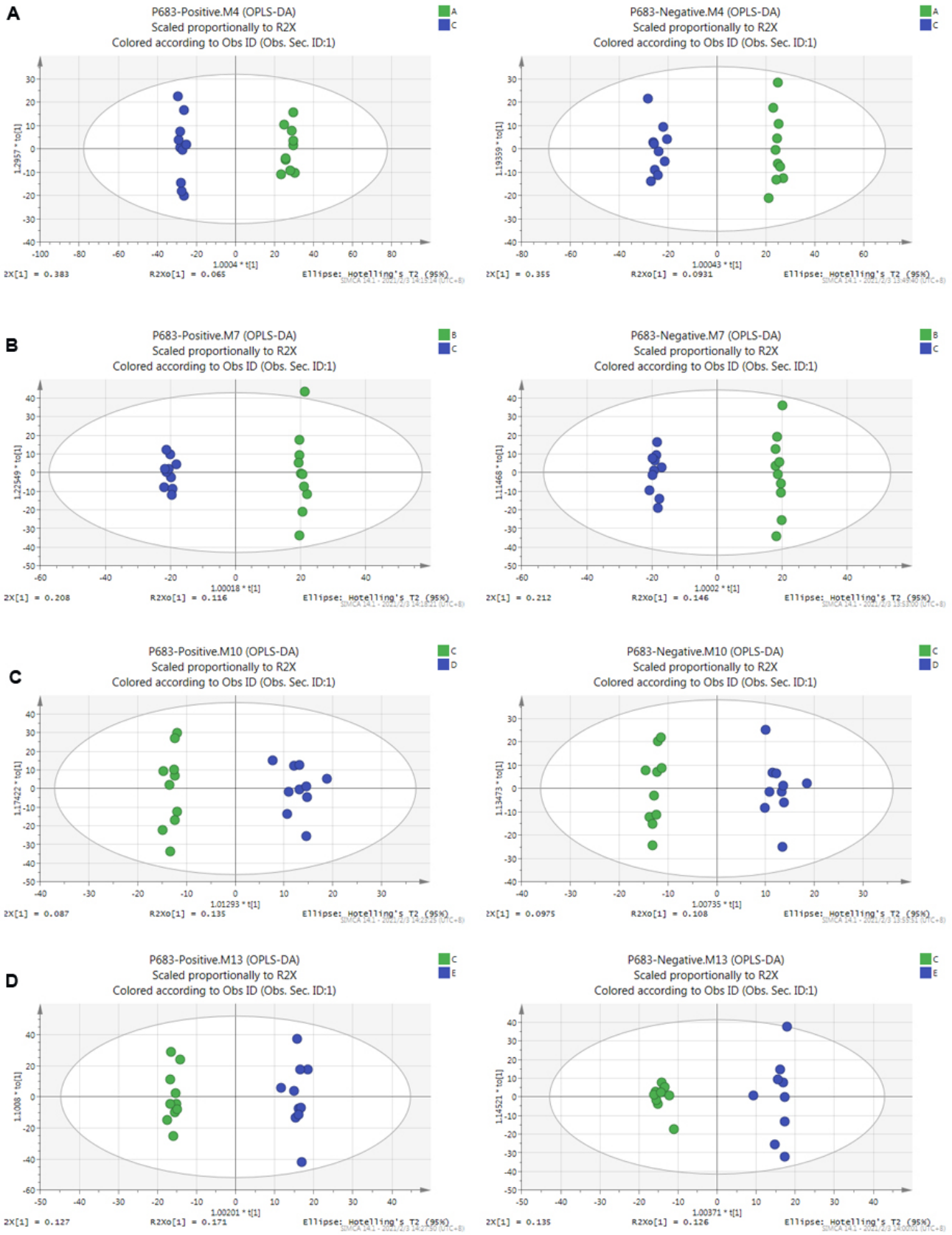

Fig. 3. Orthogonal partial least-squares discriminant analysis (OPLS-DA) scatter plots based on metabolic profiles in + and – ion modes. (A) SID group vs. NI group; (B) MID group vs. NI group; (C) MIE group vs. NI group; (D) SIE group vs. NI group. A: SID group; B: MID group; C: NI; D: MIE group and E: SIE group.

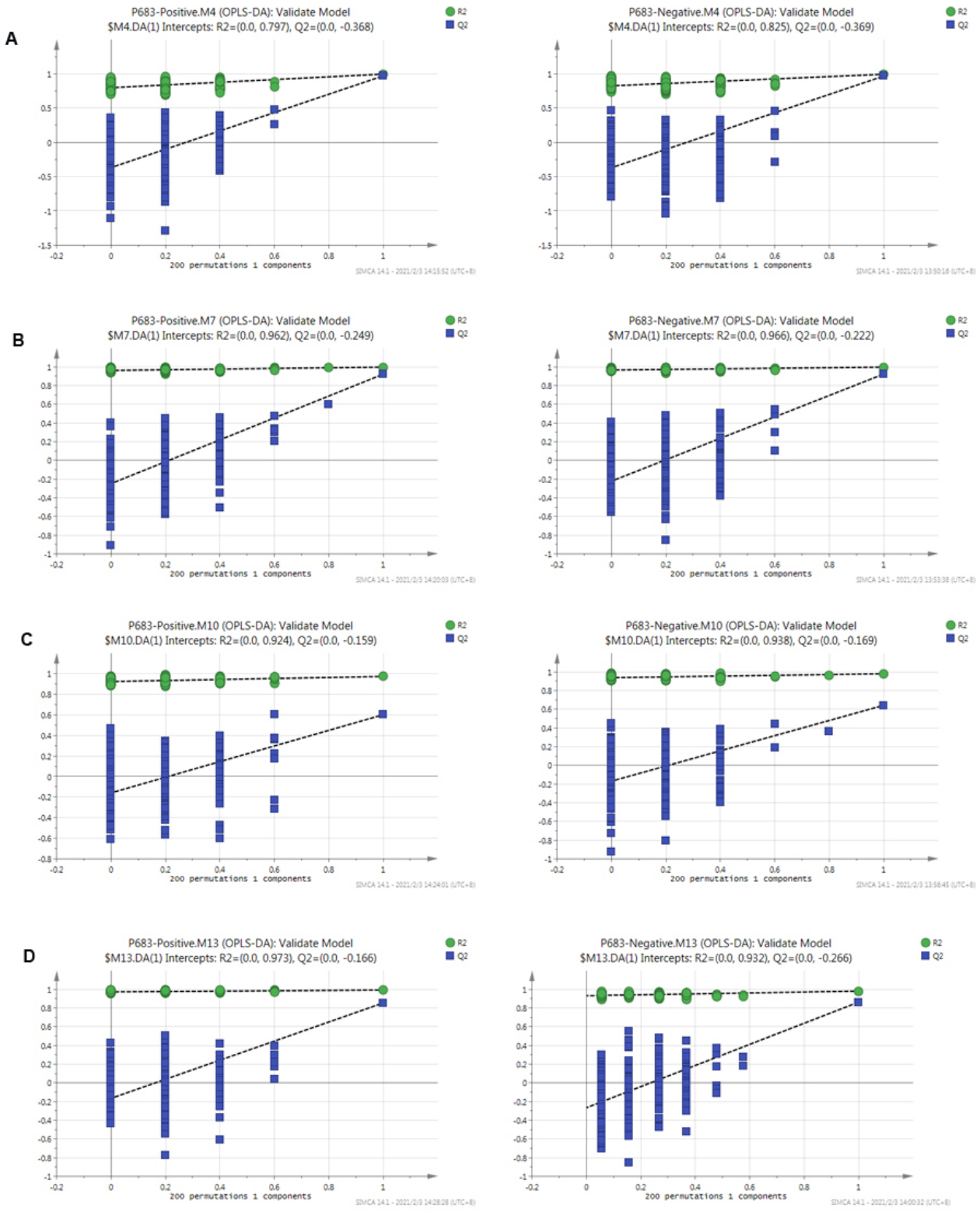

Fig. 4. The permutation tests of OPLS-DA models for statistical validation. (A) SID group vs. NI group; (B) MID group vs. NI group; (C) MIE group vs. NI group; (D) SIE group vs. NI group.

Discussion

Iodine deficiency is a global public health problem, particularly in the highland areas [22,23]. Iodine deficiency disorders remain a major public health problem that continues to affect a large proportion of the country's population [24,25]. A previous study demonstrated that different levels of iodine nutrition have important effects on pregnancy

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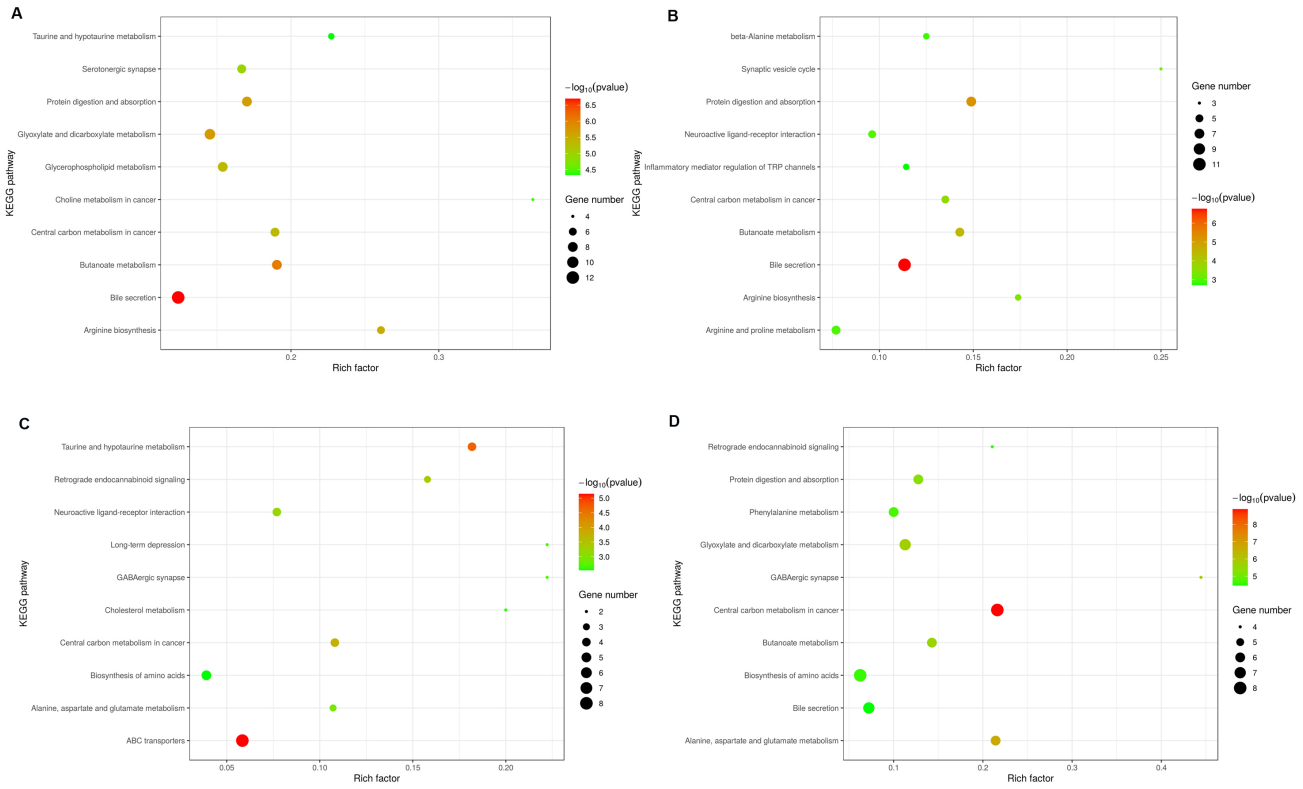

Fig. 5. Pathways enrichment analysis of differential metabolites. (A) SID group vs. NI group; (B) MID group vs. NI group; (C) MIE group vs. NI group; (D) SIE group vs. NI group.

outcomes in rats. Briefly, the number of stillbirths and miscarriages was significantly higher regardless of iodine deficiency or iodine excess. More importantly, it has been reported that the thyroid function of the mother directly affects the growth and development of the next generation [8,9]; however, the underlying mechanism is unclear. In the present study, based on the iodine excess or deficiency rat model, several metabolites, such as L-glutamic acid and deoxycytidine, as well as pathways, such as the ABC transporter pathway, which might be involved in the process of maternal iodine deficiency or excess, affected offspring development.

L-glutamate is essential for maintaining antioxidant reactions [26]. As the main oxidizing fuel in the gastrointestinal tract, L-glutamic acid is a proteogenic amino acid involved in the biosynthesis of proteins in living systems [27]. A previous study has indicated that glutamic acid can affect biochemical and physiological indices in rats with iodine deficiency [28]. In this study, the level of L-glutamic acid increased in rats with iodine deficiency. Levels of L-glutamic acid are thought to differentiate patients with papillary thyroid carcinoma (PTC) from healthy controls [29], and iodine deficiency is associated with an increased risk of developing thyroid cancer [30]. Therefore, we speculate that elevated L-glutamic acid levels in the offspring may be associated with impaired maternal thyroid function caused by iodine deficiency. Furthermore, the present study found that excess maternal iodine may cause a significant upregulation in offspring deoxycytidine levels. Deoxycytidine, also named 5-aza-2′-deoxycytidine, has been reported to have little effect on the differentiation of human thyroid cancer cell lines but modulates genes involved in adaptation *in vitro* [31]. More importantly, treatment with the epigenetic-modifying agents 5-aza-2′-deoxycytidine results in the increasing of iodine uptake in thyroid carcinoma cell lines [32]. However, the effect of excess or deficiency of iodine on the metabolic levels of deoxycytidine has not yet been reported. Among all the differential metabolites, this study also found that the levels of n-propyl GalIate, 4-(4-Nitrobenzyl)pyridine, and p-nitrophenyl-O-ethyl ethylphosphonate were significantly higher in rats with iodine deficiency but significantly decreased in rats with iodine excess. N-Propropyl gallate is a phenolic compound with antioxidant and anti-inflammatory properties [33]. 4-(4-Nitrobenzyl)pyridine can is involved in drug metabolism by binding to the cytochrome P450 [34]. Although there is no relevant literature reporting the relationship between these metabolites and iodine intake or thyroid function, our results suggest that their levels in offspring may be negatively correlated with maternal iodine intake.

Furthermore, in this study, the ABC transporter pathway was the most enriched metabolic pathway. Previous studies have shown that the ABC superfamily participates in normal and abnormal physiological processes using the

free energy of ATP hydrolysis to transport various substrates across different biofilms [35]. Emerging data indicate that the expression of ABC transporter family genes (ABCG2/BCRP) may be involved in the malignant progression of PTC cell lines [36]. ABC transporters are also involved in regulating drug resistance mechanisms in thyroid cancer [37]. This evidence suggests that there may be associations between the ABC transporter pathway and thyroid function, although the regulatory mechanism of iodine intake in the ABC transporter pathway remains unclear. GlnPQ is an ABC transporter for glutamic acid and belongs to the polar amino acids and opines family [38], while the L-glutamic acid level was significantly increased in offspring of iodine-deficient rats, suggesting that iodine deficiency may abnormally activate GlnPQ transporter-related pathways and thus enhance L-glutamic acid levels in the offspring. Deoxycytidine, another key metabolite identified in this study, targets ABC transporter A9 to induce cholesterol accumulation and p65 phosphorylation, thereby activating adaptive immunity and inhibiting tumor progression [39]. Therefore, we further speculated that excess maternal iodine may promote the expression of the ABC transporter A9 in the offspring and induce increased levels of deoxycytidine. These data indicate that maternal iodine deficiency or excess may affect the development of offspring by regulating the metabolites of L-glutamic acid and deoxycytidine via the ABC transporter pathway.

Conclusion

In conclusion, our study revealed the key metabolites and pathways involved in the effects of maternal iodine excess or iodine deficiency on offspring, which will provide insights into the pathogenesis of some congenital iodine deficiency diseases.

Availability of Data and Materials

The authors confirm that the data supporting the findings of this study are available in the article and supplementary material.

Author Contributions

Conception and design of the research: JH, CW, YZ and QL; acquisition of data: JH, CW, YZ, KP and CZ; analysis and interpretation of data: JH, CW, LZ and XC; statistical analysis: JH and CW; obtaining funding: LZ, XC and QL; drafting the manuscript: JH and CZ. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This animal experiment was approved by the Xinjiang Uygur Autonomous Region Center for Disease Control and Prevention Ethical Review Committee (No: 202002-001) and all procedures followed the Guide for the Care and Use of Laboratory Animals mandated by the National Institutes of Health.

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243805.306.

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