News Release

Title

Identification of novel neuroblastoma biomarkers using urine samples

Key Points

- **Comprehensive analysis of metabolites in urine samples obtained from children with neuroblastoma using liquid chromatography–mass spectrometry revealed three metabolic markers important for neuroblastoma diagnosis. These new biomarkers (3-methoxytyramine sulfate [3-MTS], cystathionine [CTN], and cortisol [COR]) were used for effectively distinguishing patients with neuroblastoma from the control subjects.**

- **The predictive value of the conventional biomarker, HVA/VMA, was assessed in 15 children with neuroblastoma, 2 of whom were false-negative cases. In contrast, the predictive value of 3-MTS, CTN, and COR was positive in all 15 patients with neuroblastoma.**

- **Identification of metabolic pathways specific to other cancer types and a combination of metabolites with high contribution to each cancer type may also provide useful biomarkers for cancer diagnosis.**

Summary

A research group led by Assistant Professor Kazuki Yokota and Professor Hiroo Uchida at the Department of Pediatric Surgery, Nagoya University Graduate School of Medicine, Designated Professor Akinari Hinoki at the Department of Rare and Intractable Cancer Analysis Research, with the cooperation of Hitachi, Ltd. could distinguish between 15 patients with neuroblastoma and 39 control subjects using three new biomarkers—3-methoxytyramine sulphate (3-MTS), cystathionine (CTN), and cortisol (COR).

Neuroblastoma is the most common childhood-onset solid tumor, except for brain tumors. It is related to a poor prognosis and is the second leading cause of death among all childhood cancers, with a 5-year progression-free survival rate of 30–40% for high-risk neuroblastoma. HVA/VMA is used to diagnose neuroblastoma; however, these biomarkers can sometimes lead to false-negative diagnosis.

In this study, 15 children with neuroblastoma were subjected to diagnosis using the

predictive value of the conventional HVA/VMA biomarker, and 2 of these cases were false negative. In contrast, the predictive value of the three new biomarkers, 3-MTS, CTN, and COR, was positive for all 15 patients with neuroblastoma. These results indicated that the three urinary metabolites associated with different metabolic systems identified in the present comprehensive analysis may be useful biomarkers for neuroblastoma diagnosis.

Furthermore, identification of metabolic pathways specific to other cancer types and the combination of urinary metabolites with high contribution to each cancer type may also provide valuable biomarkers for cancer diagnosis.

The results of this research were published in the international scientific journal Scientific Reports on February 18, 2021 (at 19 time in Japan).

Research Background

Childhood cancer is a rare disease, accounting for less than 1% of all cancers; however, it is the second leading cause of death among children. Therefore, developing a method for early diagnosis is desirable. In general, the mainstream cancer tests include blood tests and imaging tests (such as CT/PET), but these tests are invasive, involve pain and radiation exposure, and sometimes require sedation associated with the tests. Recent studies on liquid biopsy have focused on blood samples; however, in case of children, we believe that less invasive methods, such as urine testing, are preferable over blood testing. Because urine contains many metabolites, studies using polyamines and microRNAs have been conducted in various fields, including pediatric oncology.

For neuroblastoma diagnosis, we focused on urinary metabolites (other than HVA/VMA) that are associated with tumor characteristics and HVA/VMA. HVA/VMA biomarker is used to diagnose neuroblastoma; however, it can provide false-negative results in some cases. Therefore, we aimed at the comprehensive analysis of urinary metabolites using liquid chromatography–mass spectrometry to identify important metabolic pathways in children with neuroblastoma and to use these representative metabolites as biomarkers for accurate and early diagnosis of neuroblastoma.

Research Results

We conducted a comprehensive analysis using urine samples from children with neuroblastoma and identified 998 metabolites. After removing exogenous substances from these metabolites, we identified the tyrosine, methionine, steroid, and leucine metabolic pathways of the metabolites that were significantly increased in the neuroblastoma and control

groups.

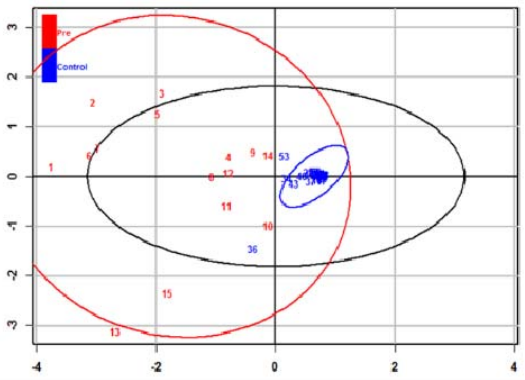
We then attempted to distinguish the neuroblastoma group from the control group using the Orthogonal Projections to Latent Structures Discriminant Analysis model. Examination of various combinations of substances with different metabolic systems confirmed that the combination of 3-methoxytyramine sulfate (3-MTS), cystathionine (CTN), and cortisol (COR), related to different metabolic systems, resulted in fewer false negatives and higher explanatory variables (R2) and predictive ability (Q2). This combination was therefore selected as the new urinary biomarker for neuroblastoma. On comparing the predictive values of the conventional tumor markers (HVA and VMA) with those of the new urinary biomarkers (3-MTS, CTN, and COR) for assessing the risk of cancer, it was observed that the former resulted in 2 false-negative cases, whereas the latter was positive for all 15 children with neuroblastoma. Positive predictive values indicate a high risk of cancer while negative predictive values indicate a low risk of cancer. The findings suggested that the levels of 3-MTS, CTN, and COR may be elevated in children aged less than 1 year.

Table1 : Nineteen of the highest-ranking metabolites according to their contributions evaluated by the random forest method.

Rank	Metabolites	Fold-change (neuroblastoma/control)	Increase or Decrease	p value	Super pathway	Sub pathway
1	homovanillate (HVA)	17	increase	<0.01	Amino Acid	Tyrosine Metabolism
2	3-methoxytyramine sulfate	12	increase	<0.01	Amino Acid	Tyrosine Metabolism
3	vanillylmandelate (VMA)	27	increase	<0.01	Amino Acid	Tyrosine Metabolism
4	vanillactate	31	increase	<0.01	Amino Acid	Tyrosine Metabolism
9	3-methoxy-4-hydroxyphenylglycol	19	increase	<0.01	Amino Acid	Tyrosine Metabolism
10	cystathionine	11	increase	<0.01	Amino Acid	Methionine Metabolism
11	3,4-dihydroxyphenyl acetate	19.	increase	<0.01	Amino Acid	Tyrosine Metabolism
13	3,4-dihydroxyphenyl acetate sulfate	9.3	increase	<0.01	Amino Acid	Tyrosine Metabolism
14	dopamine 3-O-sulfate	7.8	increase	<0.01	Amino Acid	Tyrosine Metabolism

17	dehydroascorbate	0.29	decrease	0.02	Cofactors	Ascorbate Metabolism
18	3-methoxytyrosine	13	increase	<0.01	Amino Acid	Tyrosine Metabolism
19	alpha-hydroxyisovalerate	3.6	increase	<0.01	Amino Acid	Leucine Metabolism
23	N2,N5-diacetylornithine	0.56	decrease	<0.01	Amino Acid	Arginine Metabolism
24	urea	0.77	decrease	<0.01	Amino Acid	Arginine Metabolism
25	cortisol	10	increase	<0.01	Lipid	Corticosteroids
26	3-methoxytyramine	10	increase	<0.01	Amino Acid	Tyrosine Metabolism
28	homocitrulline	0.33	decrease	<0.01	Amino Acid	Arginine Metabolism
29	tiglyl carnitine (C5)	0.77	decrease	0.018	Amino Acid	Leucine Metabolism
30	xanthurenate	0.49	decrease	<0.01	Amino Acid	Tryptophan Metabolism

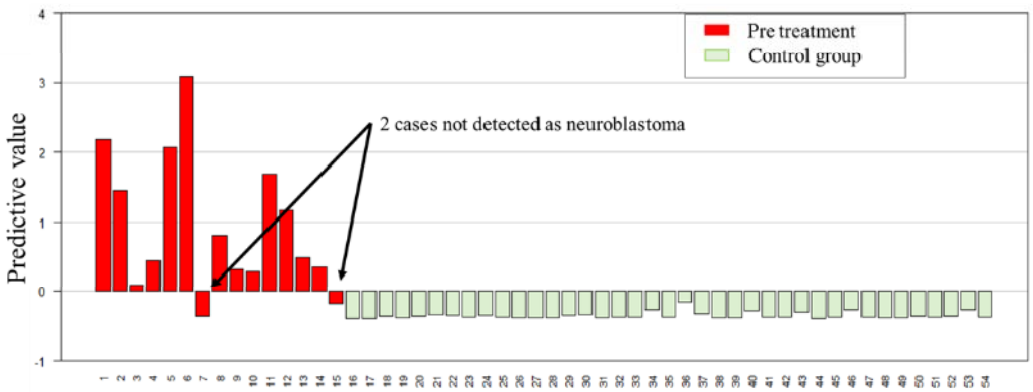
Fig. 1



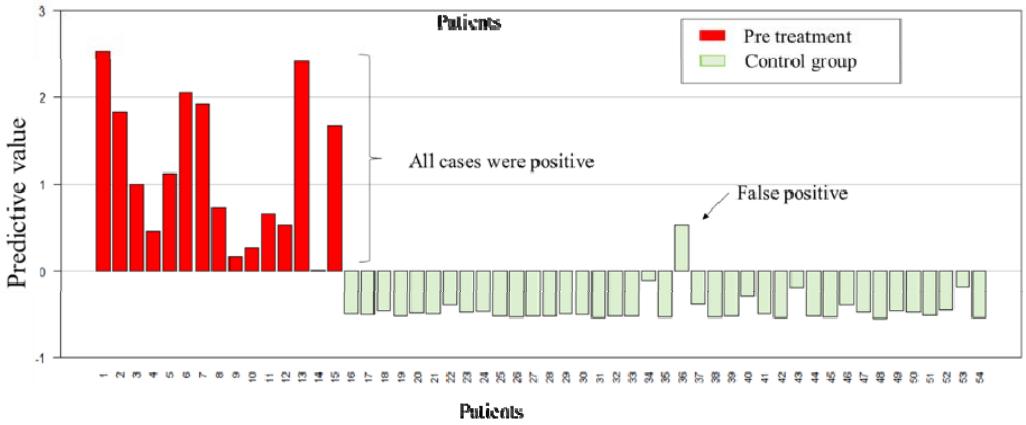
OPLS-DA results（3-MTS、CTN、COR） showed that the control group formed a very narrowly scattered pattern and the tumour group formed a widely scattered pattern.

Fig. 2

(a)



(b)



(a) Predictive value analysis of the combination of VMA and HVA .

(b) Predictive value analysis of the combinations of three metabolites （3-MTS、CTN、COR）.

Research Summary and Future Perspective

Advances in mass spectrometry and other medical devices have enabled the measurement of metabolites even at low concentrations that could not be measured in the past. In this study, to identify biomarkers other than HVA/VMA for neuroblastoma diagnosis, we performed a comprehensive analysis of urinary metabolites, identified important metabolic pathways and metabolites, and developed a method to analyze neuroblastoma risk based on the predictive value of the biomarkers. In the future, we plan to validate the urinary biomarkers of neuroblastoma identified in this study (3-MTS, CTN, and COR). In addition, this approach may be valuable in future studies to identify new biomarkers in cancer types for which no effective biomarkers have been discovered till date.

Publication

Kazuki Yokota[1], Hiroo Uchida[1*], Minoru Sakairi[2,3], Mayumi Abe[2], Yujiro Tanaka[1], Takahisa Tainaka[1], Chiyoe Shirota[1], Wataru Sumida[1], Kazuo Oshima[1], Satoshi Makita[1], Hizuru Amano[1], Akinari Hinoki[1,3]

[1] Department of Pediatric Surgery, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan.

[2] Hitachi, Ltd., R & D Group, Centre for Exploratory Research, Tokyo 185-8601, Japan

[3] Department of Rare/ Intractable Cancer Analysis Research, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

Identification of novel neuroblastoma biomarkers in urine samples, Scientific Reports Published February 18, 2021

DOI: 10.1038/s41598-021-83619-w

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Sci_Rep_210218.pdf