Iran J Public Health, Vol. 54, No.9, Sep 2025, pp.2005-2014

Original Article

Metabolomics-Based Diagnosis of Medullary Thyroid Cancer: A Plasma 1H NMR Approach

Khadijeh Saeidi [1], *Mehdi Hedayati [2], Monireh Movahedi [1], Maryam Sadat Daneshpour [1]

1. *Department of Biochemistry, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran*
2. *Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Molecular Biology, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: hedayati47@gmail.com

(Received 11 Feb 2025; accepted 27 Apr 2025)

Abstract

Background: Medullary thyroid cancer (MTC) is a rare neuroendocrine malignancy, accounting for 5-10% of all thyroid cancer cases. The precise molecular processes driving MTC remain largely elusive. We aimed to conduct a pilot study analyzing plasma metabolic profiles of MTC patients to uncover disruptions in metabolic pathways that may contribute to MTC tumorigenesis.

Methods: Proton nuclear magnetic resonance (1H-NMR) spectroscopy was performed to screen metabolic changes in plasma samples from MTC patients (n=16) and healthy subjects (n=12). Multivariate and univariate analyses were applied using MetaboAnalyst and SIMCA software.

Results: A total of 30 compounds were identified, of which three metabolites—glycerol, isobutyric acid, and valine—showed significant differences between MTC patients and the control group ($P<0.05$).

Conclusion: The findings from this study contribute to the current understanding of MTC metabolism and suggest that the NMR-based metabolomics approach can provide a metabolic pattern of MTC, potentially improving diagnostic procedures.

Keywords: Medullary thyroid cancer; Metabolomics; Diagnosis; Metabolic perturbation

Introduction

Medullary thyroid cancer (MTC) is a rare neuroendocrine malignancy, accounting for 5-10% of all thyroid cancer cases, predominantly diagnosed through fine-needle aspiration biopsy (FNAB) (1, 2). Prognosis is influenced by factors such as age, gender, tumor stage, and histological grade (3, 4). MTC can be sporadic (70-80% of cases) or inherited, with the sporadic form most commonly occurring in individuals aged 40-60 years (5). Surgical treatment, including total thyroidectomy and bilateral cervical lymph node dissection, is the primary modality for most patients (6, 7). However, the emergence of distant metastases significantly worsens prognosis, with a survival rate of 45% for patients with distant metastases at diagnosis (4, 5, 8). Thus, there is an urgent need for improved diagnostic techniques and novel therapeutic strategies for advanced stages of MTC.

Metabolomics has emerged as a powerful tool in molecular oncology, providing insights into tumor characteristics through metabolic patterns (9, 10). Various techniques, such as gas chromatog-

DOI: https://doi.org/10.18502/ijph.v54i9.19867

raphy-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), and nuclear magnetic resonance (NMR) spectroscopy, are widely used to explore metabolic alterations in diseases (11, 12). In thyroid cancer research, metabolic analyses of blood, urine, and tissue have been conducted for over two decades (13).

NMR-based metabolomics offers a valuable approach for analyzing low molecular weight metabolites across biological matrices, including plasma, urine, and tissues (14-16). Several studies have applied NMR to differentiate between thyroid cancer patients and healthy individuals by identifying altered metabolic pathways (17).

Despite these advancements, the metabolic mechanisms underlying MTC remain poorly understood at the molecular level. To address this gap, we employed untargeted 1H-NMR metabolomics to investigate plasma metabolic profiles in MTC patients, compared to healthy controls, as a preliminary step toward understanding the disease's metabolic characteristics.

Materials and Methods

Sample Collection

This case-control study was conducted among patients referred to the Cellular and Molecular Endocrine Research Center at the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences in Tehran, Iran.

Ethical approval for this study was obtained from the Ethics Committee of Shahid Beheshti University of Medical Sciences (No. IR.SBMU.ENDOCRINE.REC.1397.124). All participants, both patients and healthy controls, provided written informed consent prior to their inclusion in the study. The study adhered to the principles outlined in the Declaration of Helsinki.

Peripheral blood samples were obtained from 16 MTC patients (11 females and 5 males), with an average age of 43 ± 18.40 years. Diagnosis of MTC was confirmed through histopathological validation and clinical outcomes. Additionally, a demographically matched control group comprised 12 healthy volunteers (8 females and 4 males) with a mean age of 35.8 ± 20.95 years.

Exclusion criteria included individuals using medications that could affect thyroid function or those with other forms of malignancy and metabolic disorders, such as metabolic syndrome, diabetes, and insulin resistance (IR). Approximately 10 mL of blood was collected into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). After blood collection, plasma was promptly separated by centrifugation at 13,000 rpm for 30 minutes and stored at -80°C until analysis.

Plasma Metabolite Extraction for NMR-based Metabolomics Analysis

To extract metabolites and precipitate protein content, 1 mL of methanol was added to each 300 µL plasma sample. After vortexing, the tubes were stored at -20 °C for 20 minutes, followed by centrifugation at 13,000 rpm for 30 minutes at 4 °C. The supernatants were then transferred into new tubes and dried using an Eppendorf vacuum centrifuge for 3 hours at 45 °C. Finally, dried samples were reconstituted in a 600 µL phosphate buffer solution (composed of 0.2 M Na2HPO4/NaH2PO4) containing 10% deuterium oxide (D2O) at pH 7.4, supplemented with 5 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as a chemical shift reference (δ 0.0). After centrifugation at 13,000 rpm for 30 minutes at 4 °C, the clear supernatant was carefully transferred into a 5-mm NMR tube for NMR spectroscopy analysis.

NMR Setup

A 600 µL aliquot of each sample was subjected to 1H-NMR spectroscopic analysis, performed at a frequency of 400.202 MHz using a Bruker AscendTM 400 spectrometer (Bruker BioSpin, Germany) with the following parameters: 292 transients were acquired with 512 scans, using an 8012/82 MHz pulse width, an acquisition time of 4 s, and a repetition delay of 1 s between transients.

Statistical Data Analysis of NMR Spectra

Spectra processing was performed using the Bayesil web-based system, with the water peak at

δ 5 ppm being omitted. Phase and baseline corrections were done using Mnova NMR Suite Version 6.0.2 (Mestrelab Research, S.L., Registro Mercantil de A Coruna, Spain). The metabolites identified by Bayesil were cross-checked against the Human Metabolome Database (HMDB). Normalization and data processing were performed using MetaboAnalyst v.5.0 (https://www.metaboanalyst.ca) and SIMCA-P 14.0 software (Umetrics, Umeå, Sweden).

Orthogonal partial least squares-discriminant analysis (OPLS-DA) was utilized to identify outliers and detect metabolic differences between MTC patients and the healthy control group. Variable Importance in Projection (VIP) scores greater than 0.8 within the OPLS-DA model were considered to have significant influence in differentiating between subjects with MTC and those in the healthy group. Student's t-test ($p < 0.05$) was applied to determine significantly altered metabolites between the two groups.

Given the small sample size, a more stringent threshold was applied to increase the robustness of the findings. We applied the Benjamini-Hochberg False Discovery Rate (FDR) method to control for multiple comparisons. As a result, metabolites with an FDR-adjusted p-value of <0.05 were considered significant. This adjustment ensures that the identified metabolites have a high probability of being true positives while minimizing the risk of type I errors.

Results

The clinical and pathological characteristics of the study participants are outlined in Table 1. This study included 28 individuals divided into two groups: MTC patients (n = 16) and a healthy control group (n = 12). As shown in Table 1, the mean ages and standard deviation (SD) of MTC patients and healthy participants were (43 ± 18.40) and (35.8 ± 20.95), respectively

.

Table 1: Demographic characteristics of the study subjects

Parameter	Sex	MTC (n=16)	Healthy (n=12)	*P.* value
Sex	Male	5	4	*P*>0.999
	Female	11	8	
Age (mean ± SD; years)	Male	49.6 ± 15.70	44.25 ± 27.19	*P*= 0.7606
	Female	43.72± 14.14	35.32 ±12.82	

MTC; medullary thyroid carcinoma, SD; standard deviation

Metabolic Profiles of Plasma Samples between Two Groups by 1H NMR

Supervised analysis using OPLS-DA was conducted to identify differing metabolites between MTC patients and the healthy group. However, the OPLS-DA score plot (Fig. 1) did not show clear separation between the two groups. Hierarchical clustering heatmap analysis was performed to detect variations in metabolites between the groups (Fig. 2A).

To pinpoint metabolites with significant alterations between MTC patients and the healthy group, we combined Student's t-test analysis (*P*-value < 0.05) with a VIP score threshold > 1 derived from the OPLS-DA (Table 2). Our results revealed significant changes in three metabolites: glycerol, isobutyric acid, and valine. Box plots illustrated the mean concentrations of these three significantly altered metabolites between the MTC and healthy groups (Fig. 2B).

Table 2: Normalized concentration of metabolites in MTC and healthy patients

Metabolites	MTC (Mean ± SD)	Healthy (Mean ± SD)	*P.* value	VIP
2-Hydroxybutyrate	-0.01657389±0.202664	0.022099±0.242752	0.6621	0.738967
Acetic acid	-0.002414387±0.184436	0.003219±0.222985	0.9444	1.00382
Betaine	0.084645967±0.234102	-0.11286±0.318328	0.0798	1.48573
Acetoacetate	0.003833641±0.294702	-0.00511±0.266926	0.9371	0.504379
L-Carnitine	-0.013642358±0.220888	0.01819±0.233978	0.7258	0.929449
Creatine	-0.011785872±0.235889	0.015714±0.296182	0.7943	0.992128
Citric acid	0.031999897±0.283602	-0.04267±0.319018	0.5345	0.960428
Choline	-0.037610705±0.146675	0.050148±0.249301	0.2720	1.03563
D-Glucose	-0.023116192±0.324137	0.030822±0.364072	0.6938	0.923433
Glycine	0.006664714±0.138725	-0.00889±0.239863	0.8370	0.139075
Glycerol	-0.081695568±0.232765	0.108927±0.171019	0.0293*	1.76358
Formic acid	0.019505227±0.301707	-0.02601±0.227239	0.6766	0.507999
Hypoxanthine	-0.013363198±0.121847	0.017818±0.248022	0.6769	0.94625
L-Alanine	0.070137929±0.23085	-0.09352±0.23304	0.0865	1.28026
L-Proline	0.00679162±0.210323	-0.00906±0.221856	0.8541	0.286476
L-Threonine	0.074781489±0.303924	-0.09971±0.190974	0.1043	1.12731
L-Asparagine	0.020760723±0.230428	-0.02768±0.203474	0.5820	0.759984
L-Isoleucine	-0.005453532±0.233608	0.007271±0.179473	0.8808	0.107239
L-Lactic acid	0.05984595±0.295764	-0.07979±0.149093	0.1606	1.3331
L-Aspartic acid	-0.041052748±0.219133	0.054737±0.244627	0.3038	0.773378
Succinic acid	-0.007359423±0.187526	0.009813±0.304447	0.8608	1.0072
3-Hydroxybutyric acid	0.008124752±0.31475	-0.01083±0.18406	0.8591	0.472136
Creatinine	0.008095434±0.265566	-0.01079±0.202446	0.8445	1.03201
L-Leucine	-0.024486745±0.230767	0.032649±0.199966	0.5144	0.490737
Malonic acid	0.032523355±0.177298	-0.04336±0.310176	0.4384	1.02474
L-Methionine	0.00964029±0.230518	-0.01285±0.215053	0.8020	0.576807
Isopropanol	-0.07849301±0.306047	0.104657±0.161959	0.0809	1.2925
L-Valine	-0.066121061±0.18818	0.088161±0.171598	0.0412*	1.59705
Acetone	-0.000114847±0.240428	0.000153±0.221451	0.9977	0.330091
Isobutyric acid	-0.103479132±0.252309	0.137972±0.302099	0.0355*	1.67127

MTC; medullary thyroid carcinoma, VIP; Variable importance values in the projection, SD; standard deviation
P-values were calculated using the Student t-test. Statistical significance was defined as $p < 0.05$*. Values are presented as mean ± SD for biological replicates (MTC n=18, healthy n=12)

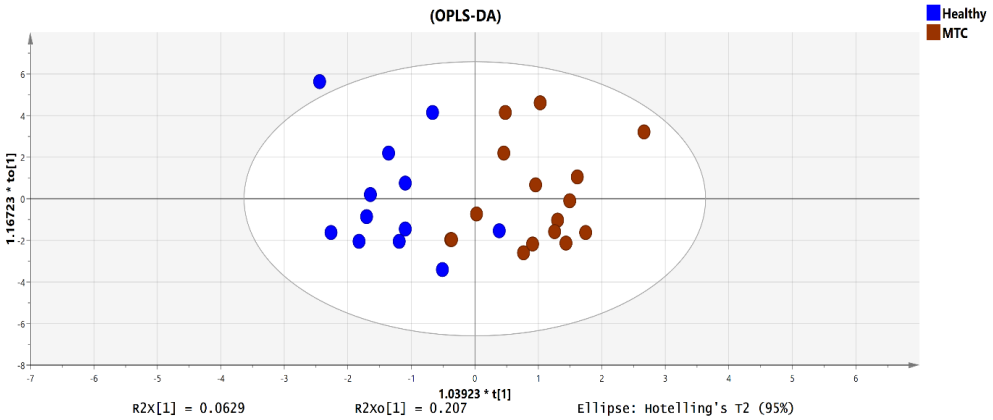

Fig. 1: OPLS-DA score scatter plot from 1H-NMR metabolic profiles of MTC (red) and healthy (blue). MTC; medullary thyroid carcinoma

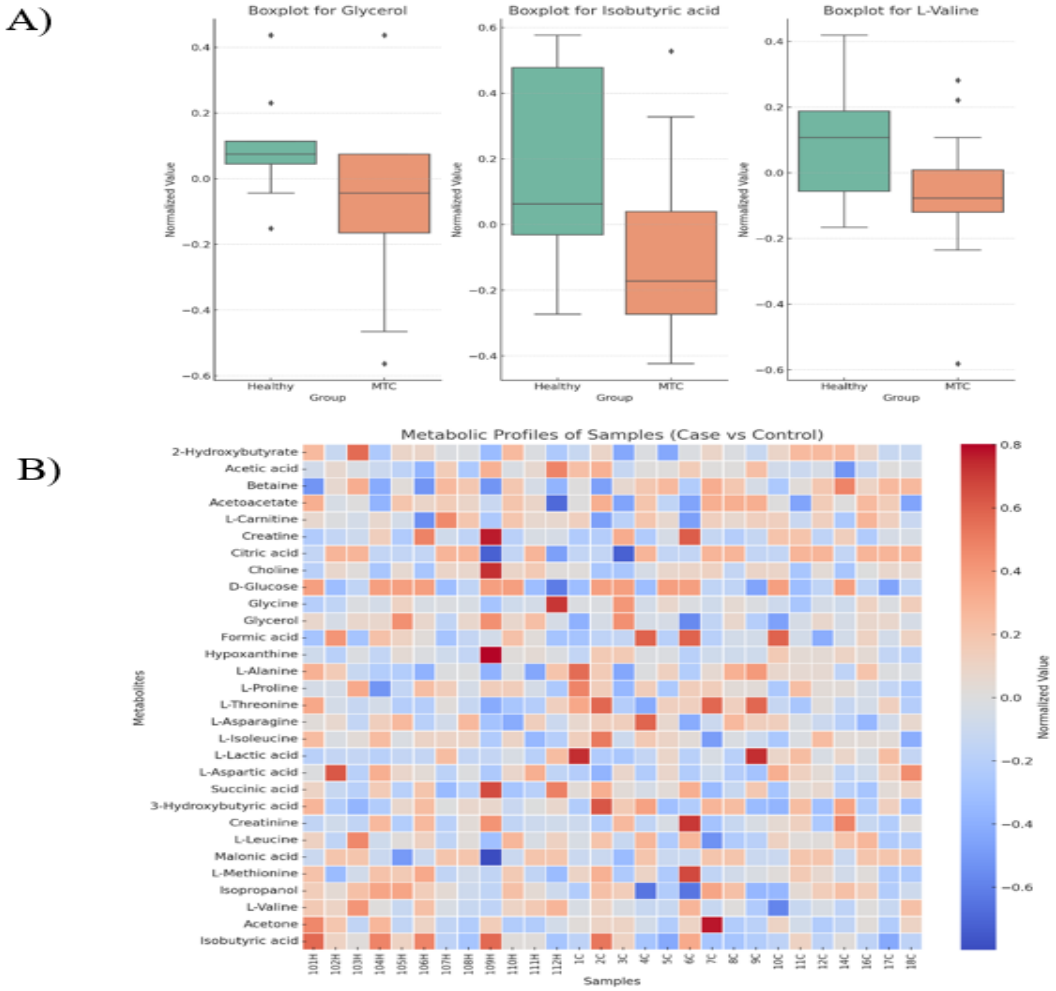

Fig. 2: A) Heatmap visualization of all metabolites. B) Box-and-whisker plots presenting the normalized values of three chosen metabolites. The specific metabolite is displayed on the x-axis, while the normalized peak intensity is displayed on the y-axis

Discussion

The main findings of our study include the identification of three metabolites—glycerol, valine, and isobutyric acid—that were significantly altered in MTC patients compared to healthy controls. These metabolites may serve as potential biomarkers for MTC diagnosis, although further validation is needed to confirm their clinical utility. While the results are promising, we acknowledge several limitations of the study. The small sample size is a key limitation, and it may have limited the statistical power of our analyses, which could explain the lack of clear separation between the MTC and control groups in the OPLS-DA plot. Additionally, the heterogeneity of MTC, with its varying metabolic profiles across patients, may have contributed to the observed variability in the data. Larger cohorts and more refined methodologies are needed to further assess the robustness and reproducibility of these findings.

Regarding the potential diagnostic utility of the identified metabolites, while their diagnostic value remains to be fully validated, these metabolites could be used in conjunction with existing diagnostic techniques, such as fine-needle aspiration biopsy (FNAB), to improve early detection and monitoring of MTC. Further studies should focus on assessing their sensitivity, specificity, and ability to distinguish MTC patients from healthy individuals, and whether they could provide complementary information alongside current diagnostic methods. The role of isobutyric acid in MTC is particularly intriguing, although its precise mechanistic role remains speculative. We hypothesize that isobutyric acid may influence key metabolic pathways involved in cancer progression, such as the tricarboxylic acid (TCA) cycle and cellular energy production. Future studies should focus on investigating these pathways to better understand the involvement of isobutyric acid in MTC and its potential as a therapeutic target or biomarker.

Thyroid cancer (TC) is the ninth most prevalent cancer in males and the fifth most prevalent in females (18). The global incidence of thyroid cancer is rising, primarily due to increased utilization and improvement of diagnostic imaging and monitoring practices (19). Despite this trend, the precise causes behind the incidence and mortality rates of TC remain partially unknown. Thus, non-invasive diagnostic biomarkers are essential in this context (20).

NMR spectroscopy is a major analytical method used in the rapidly expanding field of metabolomics. Several attributes inherent to NMR, such as its notable reproducibility, quantitative capabilities, non-selective nature, and non-invasive characteristics, make it advantageous for metabolomics applications (21). Researchers have demonstrated the crucial role of 1H-NMR metabolomics in identifying metabolic biomarkers in various diseases, including thyroid cancer (17).

In this study, we employed 1H-NMR to identify plasma metabolic alterations in MTC patients. Our findings indicated different patterns of glycerol, isobutyric acid, and valine between MTC patients and the healthy control group. While we identified three metabolites (glycerol, valine, and isobutyric acid) with significant changes between MTC patients and healthy controls, it is important to note that the p-values for these metabolites (glycerol: $p=0.0293$, valine: $p=0.0412$) are close to the threshold for statistical significance. This suggests that the findings may not be highly robust, particularly given the small sample size in this study. As such, these results should be interpreted with caution. Larger cohorts are needed to confirm these preliminary findings and assess the clinical utility of these metabolites as potential biomarkers for MTC.

Metabolic reprogramming plays a pivotal role in facilitating the proliferation of cancer cells. Essential and non-essential amino acids serve as energy sources, contribute to the biosynthesis of crucial molecules, and maintain redox

equilibrium, providing the altered metabolites necessary for cancer progression (22).

Recent advancements in understanding the tricarboxylic acid (TCA) cycle have revealed the involvement of substances beyond glucose, notably amino acids like valine and glutamine. These amino acids participate in the TCA cycle by releasing amine groups and generating acetyl-CoA. Branched-chain amino acids (BCAAs), including valine, contribute significantly to the energy production of cancer cells, accounting for approximately 12% to 55% of total ATP. The conversion of valine by valine transaminase yields glutamate, which is crucial for sustaining tumor growth and facilitating the TCA cycle (23).

In the context of thyroid cancer, J. Lu et al conducted a study involving papillary thyroid microcarcinoma (PTMC) samples (17). These investigations revealed a significant decrease in valine levels in the PTMC group. Similarly, our study demonstrated reduced valine concentration in the MTC group compared to healthy controls, potentially indicating a correlation between valine and the TCA cycle. This alteration could suggest increased protein synthesis, a process of great significance in advancing cancer.

Glycerol, another metabolite of interest in our study, is transported across cell membranes by AQP3, a transmembrane channel (24, 25). As an important metabolic molecule, glycerol serves dual roles in generating ATP (cellular energy) and producing triglycerides, both crucial for sustaining cell growth and division. Further investigation is warranted to fully understand the implications of glycerol and other altered metabolites in the context of MTC.

A study involving various thyroid cancer cell lines, including hyperplastic and neoplastic cells, revealed an intriguing pattern. MTC cells exhibited elevated expression of AQP3. This higher AQP3 expression potentially facilitates increased glycerol absorption and breakdown, leading to greater ATP production and augmented lipid synthesis. These metabolic changes collectively increase cell proliferation within MTC cells (26).

In agreement with recent research data, our results demonstrated that glycerol is critically utilized in MTC patients to provide energy and lipid synthesis necessary for robust tumor cell growth and progression. These findings underscore the complex interplay between metabolism and cancer progression, shedding light on potential avenues for therapeutic intervention in the future.

Isobutyric acid, also referred to as 2-methylpropanoic acid, belongs to the class of carboxylic acids and is classified as one of the short-chain fatty acids (SCFAs). These SCFAs have the unique capability to permeate the rumen wall and enter the bloodstream, elevating their concentrations. This, in turn, can stimulate greater secretion of growth hormone (GH) from the pituitary gland (27, 28). Consequently, isobutyric acid could influence host endocrine functions and impact diabetes and oncological diseases. For this reason, isobutyric acid could be used as a putative biomarker for various diseases, including cancer (29).

In a notable study by Wang et al., they conducted an investigation using 1H NMR-based metabolic profiles of whole blood samples obtained from healthy individuals and those diagnosed with papillary thyroid carcinoma (PTC), with or without lymph node metastasis. Their findings revealed a noteworthy increase in isobutyric acid levels among PTC patients compared to the control group. Remarkably, their research demonstrated that isobutyric acid could effectively distinguish between PTC patients and healthy individuals, although it could not discern the presence of lymph node metastasis (30). Despite the growing body of research on isobutyric acid, its role in MTC remains relatively unexplored. However, our study reveals a pivotal discovery—isobutyric acid levels are decreased in individuals diagnosed with MTC. This finding suggests that isobutyric acid plays a critical role in the development and progression of MTC and can be harnessed with a high degree of accuracy to differentiate MTC patients from healthy subjects. While the effect sizes for the three significantly altered metabolites (glycerol, isobutyric acid, and valine) are relatively small, these findings may still have clin-

ical relevance. Even modest metabolic changes can reflect important underlying biological processes, which may offer valuable insights into the pathophysiology of Medullary Thyroid Cancer (MTC). These metabolites could potentially serve as biomarkers for MTC diagnosis, especially when combined with other clinical factors or diagnostic methods. It is important to note that the clinical utility of these metabolites will require further validation in larger studies to assess their robustness and sensitivity in distinguishing MTC patients from healthy individuals (Fig. 3).

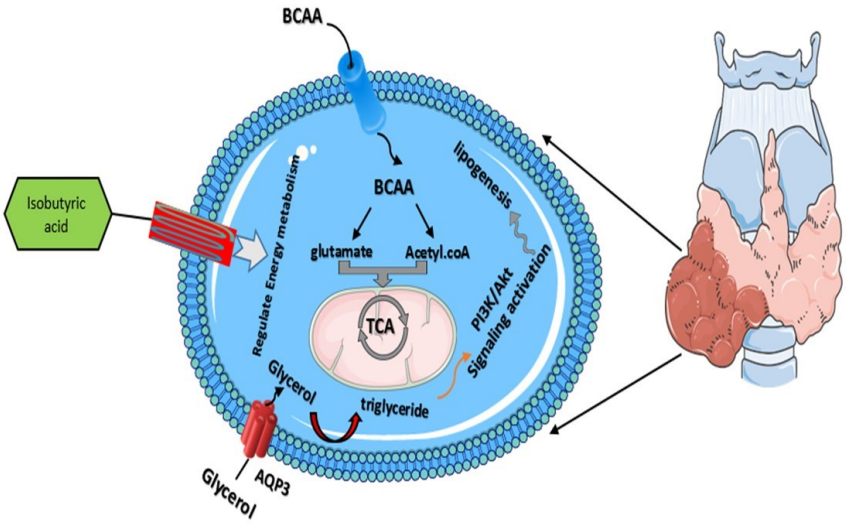

Fig. 3: Schematic form of the altered metabolite involvement in MTC. This schematic illustrates the intricate role of altered metabolites in the context of medullary thyroid cancer. The diagram visually represents the metabolic changes, such as lipogenesis, TCA, etc., within the cells of this specific type of thyroid cancer, highlighting key metabolites and their interactions. By providing a visual representation of these alterations, it helps researchers gain insights into the underlying mechanisms driving MTC, potentially leading to more effective diagnostic and therapeutic strategies. BCAA: branched-chain amino acid, TCA: tricarboxylic acid cycle, MTC: medullary thyroid carcinoma

A limitation of this study is the relatively small sample size, which is a challenge inherent to studies investigating rare diseases such as MTC. MTC accounts for approximately 5-10% of all thyroid cancers, and its low incidence and prevalence make patient recruitment difficult. While this study provides valuable preliminary data, larger case-control studies are needed to confirm and further validate these findings. We anticipate that future research with larger sample sizes will provide greater insights into the clinical utility of the identified metabolites.

Conclusion

To the best of our knowledge, this is the first study to utilize 1H-NMR-based metabolomics to investigate altered metabolites in MTC patients. Our findings unveiled distinctive metabolic reprogramming characterizing patients with MTC, primarily manifested through disruptions in valine metabolism, glycerol metabolism, and isobutyric acid metabolism—these perturbations are visually represented in Fig. 3. Further investigations conducted on a larger sample size are crucial to ascertain the sensitivity and specificity of these biomarkers. Moreover, such studies are essential to validate the practical utility of these markers in enhancing existing screening initiatives and to assess their viability for incorporation into diagnostic tests for MTC.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

We would like to thank Dr. Adeleh Razavi and Dr. Raziyeh Abooshahab for their generous support and kind advice during the course of this work.

Conflict of interest

The authors whose names are listed in this paper declare no conflict of interest with financial or non-financial subjects, matters, or materials discussed in this manuscript.

References

1. Leboulleux S, Baudin E, Travagli JP, et al (2004). Medullary thyroid carcinoma. *Clin Endocrinol (Oxf)*,61(3):299-310.
2. Zarkesh M, Zadeh-Vakili A, Azizi F, et al (2018). Altered epigenetic mechanisms in thyroid cancer subtypes. *Mol Diagn Ther*, 22:41-56.
3. Agrawal N, Jiao Y, Sausen M, et al (2013). Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. *J Clin Endocrinol Metab*, 98(2):E364-9.
4. Roman S, Lin R, Sosa JA (2006). Prognosis of medullary thyroid carcinoma: demographic, clinical, and pathologic predictors of survival in 1252 cases.*Cancer*, 107(9):2134-42.
5. Campbell MJ, Seib CD, Gosnell J (2013). Vandetanib and the management of advanced medullary thyroid cancer. *Curr Opin Oncol*, 25(1):39-43.
6. Kloos RT, Eng C, Evans DB, et al (2009). Medullary thyroid cancer: management guidelines of the American Thyroid Association.*Thyroid*, 19(6):565-612.
7. Scollo C, Baudin E, Travagli J-P, et al (2003). Rationale for central and bilateral lymph node dissection in sporadic and hereditary medullary thyroid cancer. *J Clin Endocrinol Metab*, 88(5):2070-5.
8. Ferlito A, Shaha AR, Silver CE, et al (2001). Incidence and sites of distant metastases from head and neck cancer. *ORL J Otorhinolaryngol Relat Spec*, 63(4):202-7.
9. Hutschenreuther A, Birkenmeier G, Bigl M, et al (2013). Glycerophosphoglycerol, beta-alanine, and pantothenic acid as metabolic companions of glycolytic activity and cell migration in breast cancer cell lines.*Metabolites*, 3(4):1084-101.
10. Slyshenkov VS, Rakowska M, Moiseenok AG, et al (1995). Pantothenic acid and its derivatives protect Ehrlich ascites tumor cells against lipid peroxidation. *Free Radic Biol Med*, 19(6):767-72.
11. Botros L, Sakkas D, Seli E (2008). Metabolomics and its application for non-invasive embryo assessment in IVF. *Mol Hum Reprod*, 14(12):679-90.
12. Williams R, Lenz E, Lowden J, et al (2005). The metabonomics of aging and development in the rat: an investigation into the effect of age on the profile of endogenous metabolites in the urine of male rats using 1H NMR and HPLC-TOF MS. *Mol Biosyst*, 1(2):166-75.
13. Manish Kumar G, Dev Bukhsh S, Rath S, et al (2012). Metabolic modeling and simulation analysis of thyroid disorder pathway.*J Comput Sci Syst Biol*, 5:052-61.
14. Deidda M, Piras C, Dessalvi CC, et al (2017). Distinctive metabolomic fingerprint in scleroderma patients with pulmonary arterial hypertension.*Int J Cardiol*, 241:401-6.
15. Fanos V, Cristina Pintus M, Lussu M, et al (2014). Urinary metabolomics of bronchopulmonary dysplasia (BPD): preliminary data at birth suggest it is a congenital disease. J *Matern Fetal Neonatal Med*, 27 Suppl 2:39-45.
16. Iuculano A, Murgia F, Peddes C, et al (2019). Metabolic characterization of amniotic fluids of fetuses with enlarged nuchal translucency. *J Perinat Med*, 47(3):311-8.
17. Metere A, Graves CE, Chirico M, et al (2020). Metabolomic reprogramming detected by

1H-NMR spectroscopy in human thyroid cancer tissues. *Biology (Basel)*, 9(6):112.
18. Kweon S-S, Shin M-H, Chung I-J, et al (2013). Thyroid cancer is the most common cancer in women, based on the data from population-based cancer registries, South Korea. *Jpn J Clin Oncol*, 43(10):1039-46.
19. Yu G-P, Li JC-L, Branovan D, et al (2010). Thyroid cancer incidence and survival in the national cancer institute surveillance, epidemiology, and end results race/ethnicity groups.*Thyroid*, 20(5):465-73.
20. Shabani N, Razaviyan J, Paryan M, et al (2018). Evaluation of miRNAs expression in medullary thyroid carcinoma tissue samples: miR-34a and miR-144 as promising overexpressed markers in MTC.*Hum Pathol*, 79:212-221.
21. Lu J, Hu S, Miccoli P, et al (2016). Non-invasive diagnosis of papillary thyroid microcarcinoma: a NMR-based metabolomics approach.*Oncotarget*, 7(49):81768- 81777.
22. Faubert B, Solmonson A, DeBerardinis RJ (2020). Metabolic reprogramming and cancer progression. *Science*, 368(6487):eaaw5473.
23. Vettore L, Westbrook RL, Tennant DA (2020). New aspects of amino acid metabolism in cancer. *Br J Cancer*, 122(2):150-6.
24. Chen L, Li Z, Zhang Q, et al (2017). Silencing of AQP3 induces apoptosis of gastric cancer cells via downregulation of glycerol intake and downstream inhibition of lipogenesis and autophagy. *Onco Targets Ther*, 10:2791-2804.
25. Yde J, Keely SJ, Moeller HB (2021). Expression, regulation and function of Aquaporin-3 in colonic epithelial cells. *Biochim Biophys Acta Biomembr,*1863(7):183619.
26. Niu D, Kondo T, Nakazawa T, et al (2012). Differential expression of aquaporins and its diagnostic utility in thyroid cancer. *PLoS One*, 7(7):e40770.
27. Rastelli M, Cani PD, Knauf C (2019). The gut microbiome influences host endocrine functions. *Endocr Rev*, 40(5):1271-84.
28. Liu Q, Wang C, Yang W, et al (2009). Effects of isobutyrate on rumen fermentation, lactation performance and plasma characteristics in dairy cows. *Anim Feed Sci Technol,* 154(1-2):58-67.
29. Chalova P, Tazky A, Skultety L, et al (2023). Determination of short-chain fatty acids as putative biomarkers of cancer diseases by modern analytical strategies and tools: a review. *Front Oncol*, 13:1110235.
30. Wang T, Sun Z, Wang Y, et al (2020). Diagnosis of papillary thyroid carcinoma by 1H NMR spectroscopy-based metabolomic analysis of whole blood. *Drug Discov Ther*, 14(4):187-196.