Clinica Chimica Acta 554 (2024) 117780

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

First clinical evidence that trimethylsulfonium can serve as a biomarker for the production of the signaling molecule hydrogen sulfide

Francesca Antonaros [a], Barbara Obermayer-Pietsch [b,g], Giuseppe Ramacieri [c], Beatrice Vione [c,a], Chiara Locatelli [d], Walter Goessler [d,g], Maria Caracausi [a,1], Bassam Lajin [e,f,g,1,*]

[a] Department of Biomedical and Neuromotor Sciences, Unit of Histology, Embryology and Applied Biology, University of Bologna, Via Belmeloro 8, 40126 Bologna (BO), Italy
[b] Division of Endocrinology and Diabetology, Endocrinology Lab Platform, Department of Internal Medicine and Gynecology and Obstetrics, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria
[c] Department of Medical and Surgical Science, University of Bologna, Via Massarenti 11, 40138 Bologna (BO), Italy
[d] Neonatology Unit, St. Orsola-Malpighi Polyclinic, Via Massarenti 9, 40138 Bologna, BO, Italy
[e] Institute of Chemistry, Analytical Chemistry for the Health and Environment, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria
[f] Institute of Chemistry, ChromICP, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria
[g] BioTechMed-GRAZ, 8010 Graz, Austria

ARTICLE INFO

Keywords:
Down syndrome (DS)
Trimethylsulfonium
Hydrogen sulfide
Thiosulfate
Third gaseous signaling molecule

ABSTRACT

Background: Hydrogen sulfide (H_2S) is established as the third gaseous signaling molecule and is known to be overproduced in down syndrome (DS) due to the extra copy of the CBS gene on chromosome 21, which has been suggested to contribute to the clinical manifestation of this condition. We recently discovered trimethylsulfonium (TMS) in human urine and highlighted its potential as a selective methylation metabolite of endogenously produced H_2S, but the clinical utility of this novel metabolite has not been previously investigated. We hypothesize that the elevation of H_2S production in DS would be reflected by an elevation in the methylation product TMS.

Methods: To test this hypothesis, a case-control study was performed and the urinary levels of TMS were found to be higher in the DS group (geo. mean 4.5 nM, 95 % CI 2.4–3.9) than in the control (N) group (3.1 nM, 3.5–6.0), p-value 0.01, whereas the commonly used biomarker of hydrogen sulfide, thiosulfate, failed to reflect this alteration in H_2S production (15 μM (N) vs. 13 μM (DS), p-value 0.24.

Results: The observed association is in line with the proposed hypothesis and provides first clinical evidence of the utility of TMS as a novel and more sensitive biomarker for the endogenous production of the third gaseous signaling molecule than the conventionally used biomarker thiosulfate, which is heavily dependent on bacterial hydrogen sulfide production.

Conclusion: This work shows that TMS must be explored in clinical conditions where altered metabolism of hydrogen sulfide is implicated.

1. Introduction

The view that hydrogen sulfide is merely a toxic gas has been shifting rapidly since the finding that this compound acts as a signaling molecule that serves functions as significant to human health as those of nitric oxide and carbon monoxide [1].

The third gaseous signaling molecule is produced in various tissues in the human body through the action of three enzymatic activities on cysteine, namely, cystathionine beta synthase (CBS), cystathionine gamma lyase (CSE), and cysteine aminotransferase (CAT) [2,3] (Fig. 1). In addition, it has been reported that hydrogen sulfide can also be produced from cysteine through non-enzymatic reactions involving iron and pyridoxine [4]. There is a need for a selective biomarker that predominantly reflects the levels of endogenously produced hydrogen sulfide in tissues. We previously identified the sulfur methylation product trimethylsulfonium (TMS) as a natural metabolite consistently excreted

* Corresponding author.
E-mail address: bassam.lajin@uni-graz.at (B. Lajin).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.cca.2024.117780
Received 27 October 2023; Received in revised form 3 January 2024; Accepted 10 January 2024
Available online 22 January 2024

in human urine [5]. TMS is produced by the enzyme thioether *S*-methyltransferase (also called indolethylamine N-methyltransferase) from dimethylsulfide, which can be produced through successive methylation of hydrogen sulfide [6] (Fig. 1). Therefore, TMS appears to be a promising biomarker of hydrogen sulfide. However, the utility of this compound as a biomarker in medical conditions is yet to be investigated.

Down syndrome (DS) is a common genetic disorder in which children are born with an additional copy of chromosome 21. Therefore, key genes carried on this chromosome can be overexpressed in DS and this has been demonstrated by gene-expression studies [7]. Among these genes is the *cystathionine β-synthase* (*CBS*) which encodes a pivotal enzyme for the endogenous production of hydrogen sulfide [8,9]. The resulting overproduction of hydrogen sulfide in DS is well-known [10], and has been suggested to contribute to the neurological and metabolic alterations associated with this disorder [11].

In this work, we hypothesize that overproduction of endogenous hydrogen sulfide in DS patients would lead to an increase in the production and urinary excretion of TMS, serving as first evidence for the response of TMS to a medical condition involving altered hydrogen sulfide production and supporting its utility as a hydrogen sulfide biomarker.

2. Materials and Methods

The study population consisted of 58 children with DS (age (mean ± SD): 12.7 ± 5.9 yo, 23 females and 35 males, BMI 22 ± 4) and 48 controls (14.4 ± 7.1 yo, 16 females and 32 males) including siblings of subjects with DS and without evidence of abnormal karyotype. The subjects were recruited from the Neonatology Unit, IRCCS Sant'Orsola-Malpighi Polyclinic, Bologna, Italy, in the context of routine follow up provided for DS. Residents were of European descent (i.e. Caucasian) and most were residents of the city of Bologna, Italy. The study was performed in accordance with the declaration of Helsinki and the medical ethics approval was obtained from the ethical committee of the Hospital - University of Bologna Policlinico S. Orsola-Malpighi Italy (number: 39/2013/U/Tess) and the university of Graz (GZ. 39/107/63).

Preanalytical treatment of urine samples followed standard operating procedure [12]. Urine samples were collected in a sterile plastic cup with lid and kept refrigerated at + 4 °C if immediate processing was not possible. The samples were prepared within two hours of collection by transferring to a new tube and centrifugation at 2500 g for 5 min at + 4 °C (refrigerated centrifuge). After centrifugation, filtration by 0.20 μm cut-off filter was performed to avoid contamination of the metabolome with soluble molecules derived from cellular components. The prepared urine was transferred to sterile cryovials in 1.0 mL aliquots and stored at −80 °C until analysis.

Urine was analyzed for TMS, thiosulfate, and cystine according to the method developed and validated as previously described [13]. Briefly, the method was based on liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UHPLC-ESIMS/MS). An Agilent 1260 Infinity II LC system was employed for chromatographic separation performed on a Zorbax Eclipse Plus C18 RRHD column (50 mm × 2.1 mm, 1.8 μm particle size. Detection was performed using a triple quadrupole Ultivo® LC/TQ system (Agilent Technologies, Waldbronn, Germany) operated in the multiple reaction monitoring mode [13].

After thawing at room temperature, urine samples were mixed and filtered through a 13 mm Nylon 66 syringe filter with a pore size of 0.22 μm (BGB Analytik GmbH, Germany), before transfer to 0.7 mL polypropylene HPLC vial and injection into the HPLC system. For TMS and cystine, the mobile phase consisted of 95 % of 0.2 % (v/v) heptafluorobutyric acid and 5 % acetonitrile. For thiosulfate, a mobile phase containing 90 % of 0.2 % (v/v) heptafluorobutylamine (adjusted to pH 4.0 with formic acid) and 10 % methanol was used for isocratic separation.

Isotopically labelled internal standards were employed to compensate for matrix effects, namely, deuterated cystine (Cys-d_4), deuterated TMS (TMS-d_6), and thiosulfate-^{34}S. Solutions of the isotopically labeled internal standards were co-injected with the samples and standards using the LC autosampler. Mass transitions corresponding to qualifier and quantifier ions of the target analytes and the isotopically labeled internal standards were monitored as previously described [13].

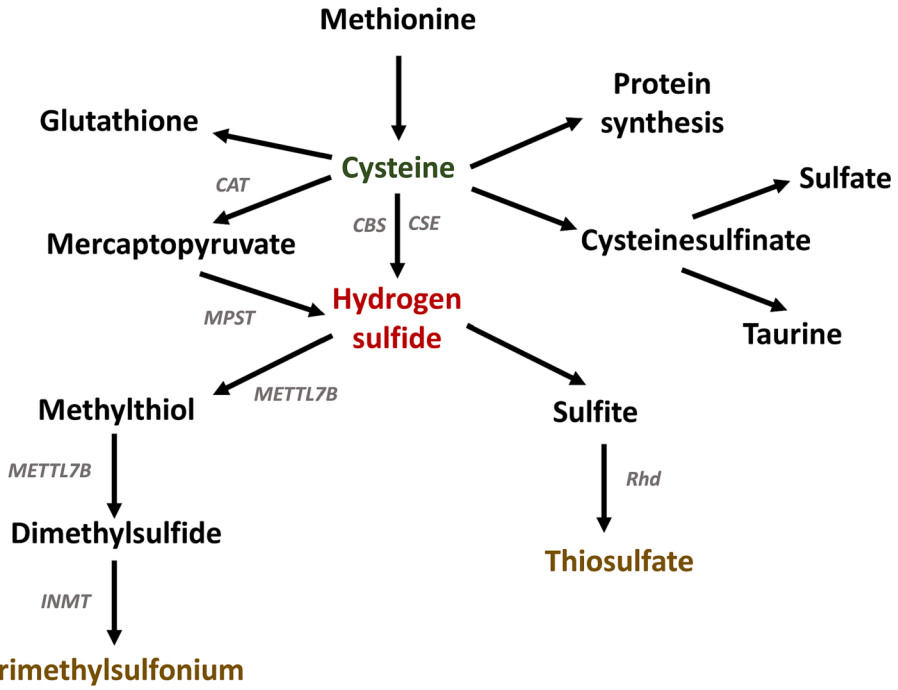

Fig. 1. Hydrogen sulfide metabolism in humans. Three enzymatic activities contribute to the production of hydrogen sulfide from the amino acid cysteine in humans. Excess hydrogen sulfide is oxidized or methylated. 3-MPST, 3-mercaptopyruvate sulfurtransferase; CAT, cysteine aminotransferase; CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; INMT, Indoleethylamine N-methyl transferase; METTL7B, methyltransferase-like 7B (thiol S-methyltransferase).

The trimethylselenonium (TMSe) production phenotype was determined by measuring urinary concentrations of TMSe with HPLC-ICPMS/MS. A YMC Triart C18 column (50 mm X 2.1 mm) was used for chromatographic separation performed in isocratic mode using a mobile phase composed of 20 % of 1,2 hexanediol, 40 % of 1 % v/v perfluorohexanoic acid pH 9.0 (adjusted with ammonia) and 40 % water at a mobile phase flow rate of 0.4 mL min^{-1} and column temperature of 40° C. Element-selective detection was performed using Agilent 8900 ICPMS/MS system (Waldbronn, Germany) at m/z 80 → 96 using oxygen as a reaction gas (0.3 mL min^{-1}). The limit of detection (LOD) was 0.05 μg Se L^{-1} and TMSe non-producers, denoted by (-) in the present report, did not show detectable levels, whereas all TMSe producers (+) (ca. 25

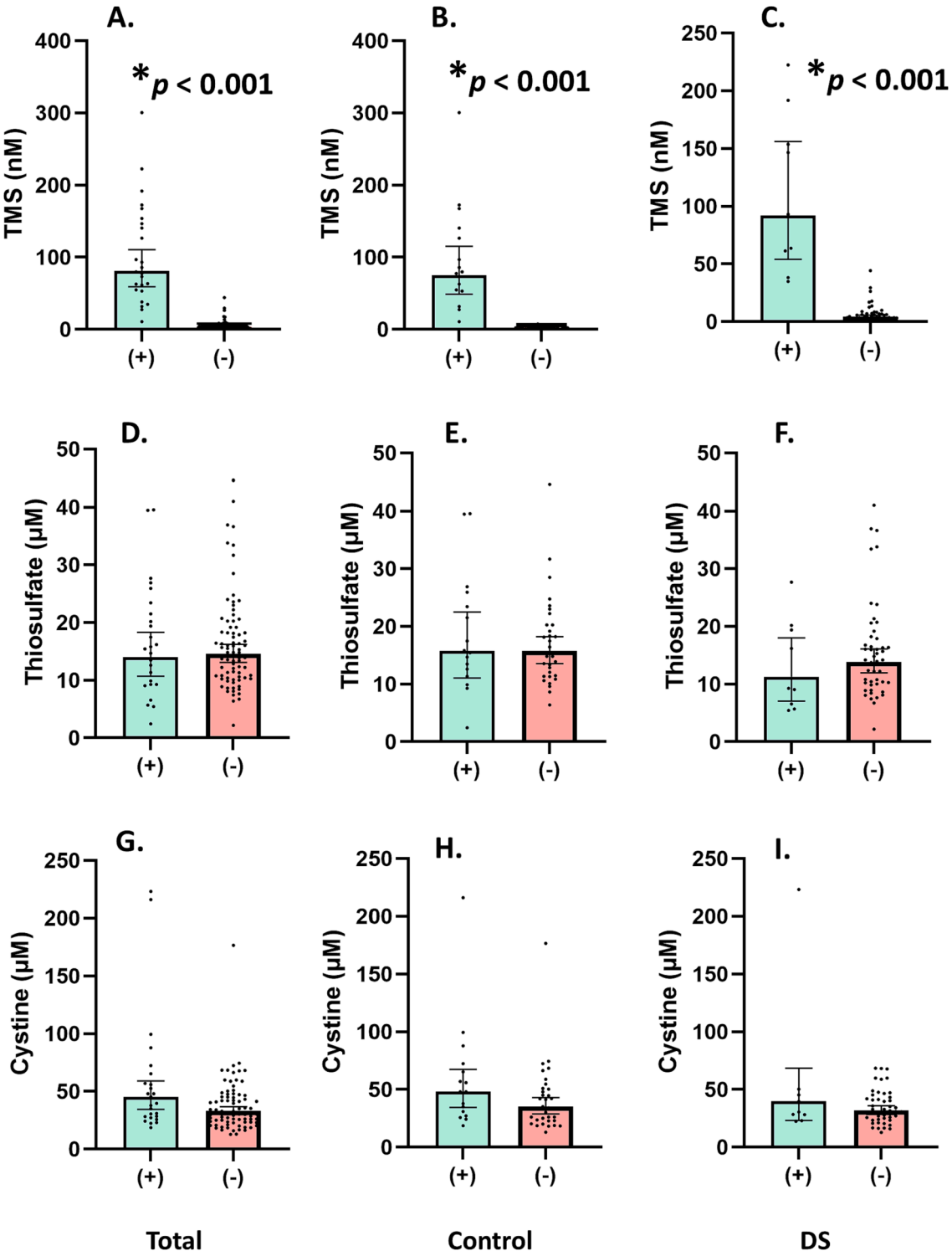

Fig. 2. The influence of trimethylsulfonium (TMS) production phenotype on the urinary levels of the investigated metabolites. The trimethylselenonium (TMSe) status is indicated by (+) for TMSe producers and (-) for TMSe non-producers. The data is shown as the geometric mean and its confidence interval (95 % CI). Individual points represent concentrations in the individual samples measured, adjusted to specific gravity. Graphs A, D, G represent comparisons based on the total pool of samples. Graphs B, E, and H represent control samples and graphs C, F, I represent Down syndrome samples.

% of the overall study population) displayed urinary levels > 20-fold above LOD, generally > 1.0 μg Se L^{-1}.

To account for variability in fluid intake, all measured concentrations were adjusted according to specific gravity which serves as a simple and equally reliable alternative to creatinine measurement [14].

3. Results

The urinary concentrations of the three metabolites under investigation in the present study were statistically examined. The data was found to follow a lognormal distribution and therefore urinary concentrations were transformed into their log values before performing statistical tests that assume normality. Furthermore, the assumption of equal variances was examined with an *F*-test and unequal variances were found between the DS and control groups for TMS (*p*-value 0.047) and therefore in this case a Welch's corrected *t*-test was used instead of a *t*-test.

The association between TMSe status (denoted by (+) for TMSe producers and (-) for TMSe non-producers, for details see discussion section) and the urinary levels of cystine, thiosulfate, and TMS was

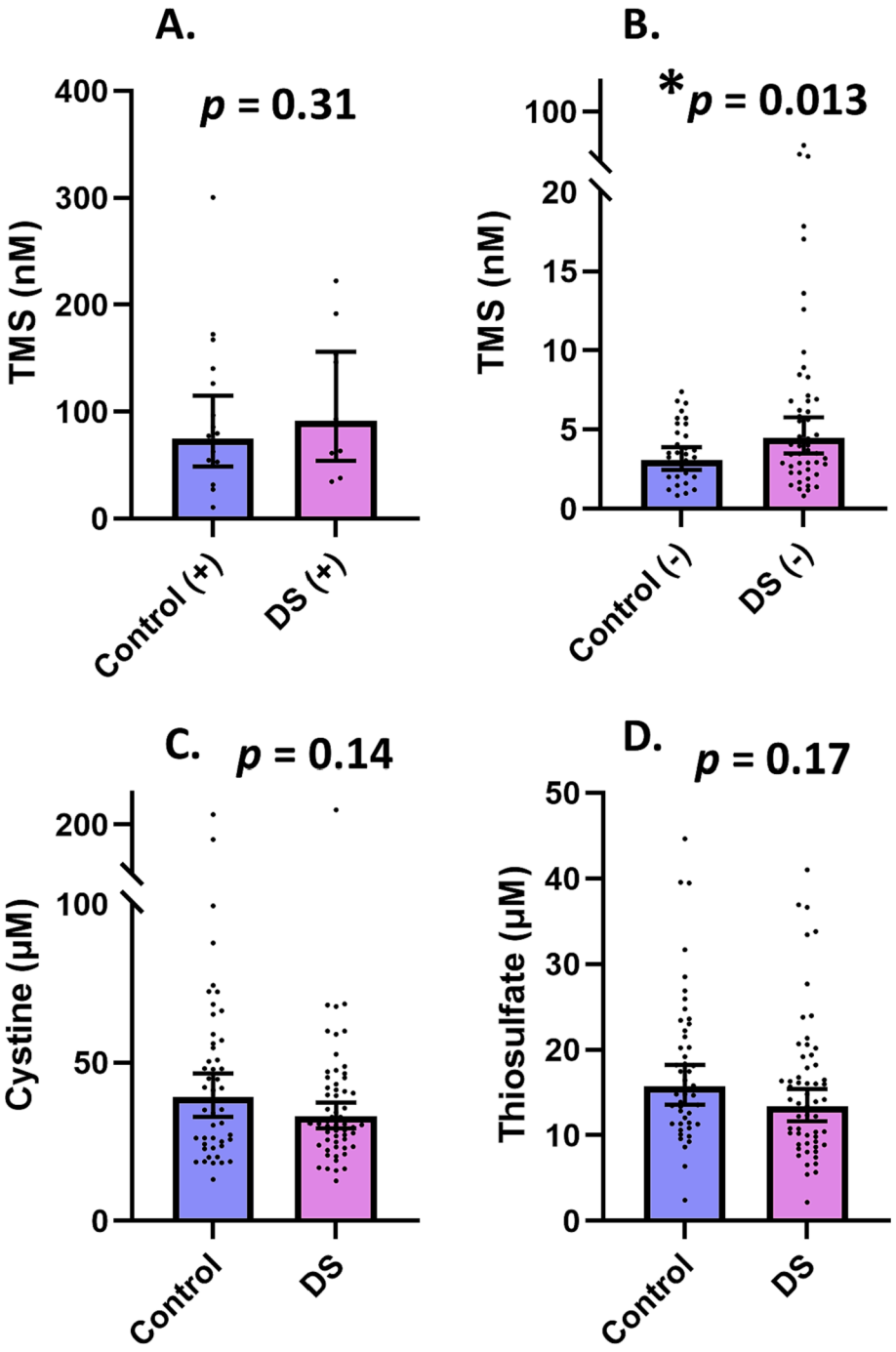

Fig. 3. The urinary concentrations of trimethylsulfonium (TMS), cystine, and thiosulfate in the Down syndrome and control groups. For trimethylsulfonium, the controls and Down syndrome (DS) patients were classified according to the TMSe production phenotype indicated by (+) and (-), for TMSe producers, and TMSe non-producers, respectively. The data is shown as the geometric mean and its confidence interval (95 % CI). Individual points represent concentrations in the individual samples measured, adjusted to specific gravity. The difference between geometric means in the control and DS group with regard to the TMSe low producers subgroup is statistically significant (one-tailed p-value 0.013, Welch's corrected *t*-test).

investigated (Fig. 2). A clear association between TMS levels and TMSe status was observed in both groups (control and DS) as well as in the total pool of samples (Fig. 2A-C), p-value < 0.001, whereas no association was found for thiosulfate and cystine (Fig. 2D-I).

Therefore, the subjects in DS and control groups were sub-grouped according to their TMSe production phenotype (Fig. 3A, B). In both subgroups, the urinary levels of TMS were higher in the DS group than in the control group (see Supplementary Table S1). However, the difference was statistically significant only in the larger size TMSe non-producers (-) subgroup (one-tailed p-value 0.013, Welch's corrected t-test). By contrast, no statistically significant association was found between the urinary levels of thiosulfate or cystine and down syndrome (Fig. 3C, D).

4. Discussion

In their search for the enzyme responsible for the production of TMSe, which was at the time thought to be the primary detoxification product of selenium in mammals including humans, Mozier et al. isolated the enzyme thioether S-methyltransferase (also called indolethylamine N-methyl transferase) from mouse lung [15] and demonstrated its capability to add a third methyl group to the dimethylated forms of three elements which belong to group 6A of the periodic table and therefore share similar chemical properties, namely, dimethylsulfide, dimethylselenide, and dimethyltelluride, converting these to the cations trimethylsulfonium (TMS), trimethylselenonium (TMSe), and trimethyltelluronium (TMTe).

Although the occurrence of TMSe in human urine was well-known [16,17], the presence of TMS in human urine was not previously reported. In 2016, we developed a sensitive analytical method to establish its presence in humans and found that this metabolite is produced naturally and excreted consistently at low nanomolar concentrations in human urine [5]. The exact origin of TMS in humans is unknown. However, given that the precursor dimethylsulfide can arise from successive methylation of hydrogen sulfide through the action of thiol S-methyltrasferases [18], it is plausible that TMS can be a biomarker of hydrogen sulfide levels.

Major inter-individual variability (up to 100-fold) in the urinary levels of TMSe has been consistently observed over the past two decades and humans were classified into two distinct groups referred to as "TMSe producers" and "TMSe non-producers" with a ratio of about 1:3 in the European population [19]. This variability has been largely explained by genetic polymorphisms in the gene encoding the enzyme responsible for the production of TMSe and TMS, thioether S-methyl transferase (also called indolethylamine N-methyltransferase, INMT, NCBI Gene ID: 11185) [20]. Indeed, we previously found that the TMSe phenotype was also associated with inter-individual variability in TMS production, even though the variability was less profound for TMS (5–10 fold) as that for TMSe [21]. For this reason, any attempt to interpret the levels of TMS as a biomarker must consider this genetic variability and therefore we determined the TMSe phenotype in the current study according to the method previously described [21]. In accordance with our previous report, the urinary levels of TMS were ca. 10-fold higher in the "TMSe producers" than that in the "TMSe nonproducers" group (Supplementary Table S1). However, the TMSe phenotype did not influence the urinary levels of the oxidation biomarker of hydrogen sulfide thiosulfate or the precursor of natural hydrogen sulfide production cystine (Fig. 2).

After subgrouping according to the TMSe phenotype, the urinary levels of TMS in the Down syndrome group were higher than those in the control group (Fig. 3A & B) and the results were statistically significant (one-tailed p-value 0.013, Welch's corrected t-test) in the larger "TMSe non-producers" group (Fig. 3B). This is in line with our hypothesis that higher hydrogen sulfide production in DS due to the extra copy of the *CBS* gene on the 21st chromosome can lead to higher TMS production. This is the first clinical evidence of the response of TMS to a medical condition involving altered hydrogen sulfide metabolism. Moreover, elevated hydrogen sulfide in DS has been reported to contribute to the clinical features of this condition [11] and it is plausible to assume that the above-mentioned inter-individual variability in TMS production may lead to inter-individual variability in hydrogen sulfide accumulation which may in turn lead to inter-individual variability in the clinical presentation in DS.

This is not the first time a hydrogen sulfide biomarker is investigated in DS. Belardinelli et al., reported the urinary levels of the conventional hydrogen sulfide biomarker thiosulfate in 17 DS patients and 17 controls, and indeed a statistically significant 2-fold increase in thiosulfate in DS was observed [22]. However, this is in contrast with the findings in the present study which included a larger sample size, where similar urinary levels of thiosulfate were observed between the DS (13 μM) and control (16 μM) groups, and the results were not statistically significant (p-value 0.17).

A closer look at the production routes for hydrogen sulfide in humans may explain the discrepancy in response between TMS and thiosulfate in DS. The human colon is colonized by sulfate-reducing bacteria which is responsible for high level of hydrogen sulfide exposure [23]. High enzymatic activity of sulfide oxidation to thiosulfate serves as the main detoxification mechanism against this exposure and the micromolar concentrations of this oxidation biomarker (Supplementary Table S1) in urine are in line with the previously reported micromolar concentrations of free hydrogen sulfide (up to 40 μM [23]) in the colon. In contrast, methylation activity of hydrogen sulfide in the colonic mucosa was reported to be low [24]. Therefore, thiosulfate appears to reflect the bacterial exposure to hydrogen sulfide to a far greater extent than TMS. In fact, TMS levels in urine occur in the nanomolar range (Supplementary Table S1) which is interestingly commensurate with the previously reported steady-state nanomolar concentrations of hydrogen sulfide in mammalian tissues (10–20 nM in mouse liver and brain) [24]. In other words, it can be speculated that TMS is a more selective biomarker of enzymatically produced hydrogen sulfide which acts as a gaseous signaling molecule in tissues.

In conclusion, the present study shows the first evidence of the response of TMS to clinical overproduction of hydrogen sulfide in humans. The discrepancy in behavior between TMS and the conventional biomarker thiosulfate suggests that TMS might be a more sensitive and selective biomarker. Future work will involve establishing the utility in various medical conditions other than DS involving altered hydrogen sulfide biochemistry.

Ethical approval and participant consent.

The study was performed in accordance with the declaration of Helsinki and the medical ethics approval was obtained from the ethical committee of the Hospital - University of Bologna Policlinico S. Orsola-Malpighi Italy (number: 39/2013/U/Tess) and the university of Graz (GZ. 39/107/63).

CRediT authorship contribution statement

Francesca Antonaros: Investigation, Methodology, Writing – review & editing. **Barbara Obermayer-Pietsch:** Data curation, Investigation, Writing – review & editing. **Giuseppe Ramacieri:** Formal analysis, Investigation, Writing – review & editing. **Beatrice Vione:** Data curation, Formal analysis, Writing – review & editing. **Chiara Locatelli:** Data curation, Formal analysis, Writing – review & editing. **Walter Goessler:** Supervision, Writing – review & editing. **Maria Caracausi:** Conceptualization, Supervision, Writing – review & editing. **Bassam Lajin:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment and role of funding source

FA fellowship has been funded by the European Union - NextGenerationEU - Alma Idea 2022 - Alma Mater Studiorum University of Bologna. Trisomy 21 research of the Bologna group is supported by Fondazione Umano Progresso, Milan and Fondazione Rosa Prístina, Pisa.

This research was funded by the Austrian Science Fund (FWF) [FWF P 32379]. For the purpose of open access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2024.117780.

References

[1] D.J. Lefer, A new gaseous signaling molecule emerges: cardioprotective role of hydrogen sulfide, Proc. Natl. Acad. Sci. 104 (2007) 17907–17908.
[2] B. Renga, Hydrogen sulfide generation in mammals: the molecular biology of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), Inflamm. Allergy Drug, Targets 10 (2011) 85–91.
[3] N. Shibuya, H. Kimura, Production of hydrogen sulfide from d-cysteine and its therapeutic potential, Front. Endocrinol. (lausanne) 4 (2013) 87.
[4] J. Yang, P. Minkler, D. Grove, R. Wang, B. Willard, R. Dweik, C. Hine, Non-enzymatic hydrogen sulfide production from cysteine in blood is catalyzed by iron and vitamin B6, Commun. Biol. 2 (2019) 194.
[5] B. Lajin, K.A. Francesconi, The hydrogen sulfide metabolite trimethylsulfonium is found in human urine, Sci. Rep. 6 (2016) 1–6.
[6] D.A. Russell, M.K. Chau, Y. Shi, B.J. Maldonato, R.A. Totah, METTL7A and METTL7B are responsible for S-thiol methyl transferase activity in liver, bioRxiv 2022 (10) (2022).
[7] M.C. Pelleri, C. Cattani, L. Vitale, F. Antonaros, P. Strippoli, C. Locatelli, G. Cocchi, A. Piovesan, M. Caracausi, Integrated quantitative transcriptome maps of human trisomy 21 tissues and cells, Front. Genet. 9 (2018) 125.
[8] M. Dierssen, Down syndrome: the brain in trisomic mode, Nat. Rev. Neurosci. 13 (2012) 844–858.
[9] A. Mégarbané, A. Ravel, C. Mircher, F. Sturtz, Y. Grattau, M.-O. Rethoré, J.-M. Delabar, W.C. Mobley, The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome, Genet. Med. 11 (2009) 611–616.
[10] C. Szabo, The re-emerging pathophysiological role of the cystathionine-β-synthase-hydrogen sulfide system in Down syndrome, FEBS J. 287 (2020) 3150–3160.
[11] P. Kamoun, Mental retardation in Down syndrome: a hydrogen sulfide hpothesis, Med. Hypotheses 57 (2001) 389–392.
[12] P. Bernini, I. Bertini, C. Luchinat, P. Nincheri, S. Staderini, P. Turano, Standard operating procedures for pre-analytical handling of blood and urine for metabolomic studies and biobanks, J. Biomol. NMR 49 (2011) 231–243.
[13] B. Lajin, B. Obermayer-Pietsch, W. Goessler, Rapid determination of hydrogen sulfide-related metabolites in human urine by UHPLC-ESI-MS/MS with fluorinated ion-pairing reagents, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1196 (2022) 123198.
[14] J.E. Muscat, A. Liu, J.P. Richie Jr, A comparison of creatinine vs. specific gravity to correct for urinary dilution of cotinine, Biomarkers 16 (2011) 206–211.
[15] N.M. Mozier, K.P. McConnell, J.L. Hoffman, S-adenosyl-L-methionine: thioether S-methyltransferase, a new enzyme in sulfur and selenium metabolism, J. Biol. Chem. 263 (1988) 4527–4531.
[16] N. Oyamada, M. Ishizaki, Determination of trimethylselenonium ions in human urine by graphite furnace atomic absorption spectrometry, Sangyo Igaku 24 (1982) 320–321.
[17] A.T. Nahapetian, V.R. Young, M. Janghorbani, Measurement of trimethylselenonium ion in human urine, Anal. Biochem. 140 (1984) 56–62.
[18] B.J. Maldonato, D.A. Russell, R.A. Totah, Human METTL7B is an alkyl thiol methyltransferase that metabolizes hydrogen sulfide and captopril, Sci. Rep. 11 (2021) 1–13.
[19] D. Kuehnelt, D. Juresa, N. Kienzl, K.A. Francesconi, Marked individual variability in the levels of trimethylselenonium ion in human urine determined by HPLC/ICPMS and HPLC/vapor generation/ICPMS, Anal. Bioanal. Chem. 386 (2006) 2207–2212.
[20] D. Kuehnelt, K. Engström, H. Skröder, S. Kokarnig, C. Schlebusch, M. Kippler, A. Alhamdow, B. Nermell, K. Francesconi, K. Broberg, Selenium metabolism to the trimethylselenonium ion (TMSe) varies markedly because of polymorphisms in the indolethylamine N-methyltransferase gene, Am. J. Clin. Nutr. 102 (2015) 1406–1415.
[21] B. Lajin, K.A. Francesconi, The Association between the Urinary Excretion of Trimethylselenonium and Trimethylsulfonium in Humans, PLoS One 11 (2016) e0167013.
[22] M.-C. Belardinelli, A. Chabli, B. Chadefaux-Vekemans, P. Kamoun, Urinary sulfur compounds in Down syndrome, Clin. Chem. 47 (2001) 1500–1501.
[23] F. Suarez, J. Furne, J. Springfield, M. Levitt, Production and elimination of sulfur-containing gases in the rat colon, American Journal of Physiology-Gastrointestinal and Liver, Physiology 274 (1998) G727–G733.
[24] M.D. Levitt, J. Furne, J. Springfield, F. Suarez, E. DeMaster, Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa, J. Clin. Invest. 104 (1999) 1107–1114.