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ORIGINAL ARTICLE

Urinary metabolomic profiling in 22q11.2 deletion syndrome reveals microbial and mitochondrial signatures related to autism and psychosis risk

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Abstract

Aim: 22q11.2 deletion syndrome (22qDS) is the most common copy-number-variation disorder, associated with multi-organ anomalies and elevated risk for schizophrenia and other neuropsychiatric conditions. Previous metabolomic studies have used blood samples, implicating mitochondrial dysfunction and amino acid imbalance, but no urinary metabolomic analysis has been reported. We aimed to characterize the urinary metabolomic profile of 22qDS.

Methods: We conducted an exploratory study comparing urine from 10 individuals with 22qDS and 10 age- and sex-matched healthy controls. Metabolites were quantified using capillary electrophoresis time-of-flight mass spectrometry and liquid chromatography time-of-flight mass spectrometry. Data were analyzed using principal component analysis and Wilcoxon rank-sum tests with false-discovery-rate adjustment.

Results: Principal component analysis indicated separation between groups. Several metabolites differed significantly, defined by a false discovery rate $q < 0.20$ and fold change > 1.5 or <0.67. Elevated metabolites in 22qDS included 2-hydroxyglutaric acid,

p-cresol sulfate, *p*-cresol glucuronide, trimethylamine-*N*-oxide, and 3-indoxylsulfuric acid, whereas citrulline and lysine were reduced. These metabolites are implicated in mitochondrial dysfunction, amino acid imbalance, and gut microbial dysbiosis. A substantial proportion of altered metabolites corresponded to those previously reported in autism spectrum disorder (ASD), predominantly microbiota-related.

Conclusion: This first urinary metabolomic study of 22qDS demonstrates systemic metabolic alterations, including mitochondrial and microbiota-associated changes. The overlap with ASD is suggestive of a possible shared metabolic signature. Our findings provide initial insights into systemic and microbial contributions to neuropsychiatric vulnerability in this genetically defined high-risk population.

KEYWORDS

22q11.2 deletion syndrome, dysbiosis, mitochondrial dysfunction, psychotic disorders, urinary metabolomics

INTRODUCTION

22q11.2 deletion syndrome (22qDS) is the most common copy-number-variation disorder involving microdeletions (approximately 0.7–3.0 Mb) on chromosome 22q11.2, with an estimated prevalence of 1 in 3000–6000 live births. It presents with a heterogeneous spectrum of multi-organ clinical features, including congenital heart anomalies, palatal defects, immune dysfunction, and hypocalcemia. In addition to various physical manifestations, 22qDS is a highly penetrant genetic risk factor for psychotic disorders, such as schizophrenia. Approximately 25% of the individuals with 22qDS develop schizophrenia compared with a prevalence of 0.5%–1% in the general population.[1] Furthermore, the lifetime prevalence of any schizophrenia spectrum disorder is 41% in individuals with 22qDS aged > 25 years.[2] 22qDS is also associated with neuropsychiatric comorbidities, including intellectual disability (~50%), attention-deficit/hyperactivity disorder (30%–55%), autism spectrum disorder (ASD) (14%–50%), and anxiety disorders (~50%).[3] The 22q11.2 deletion is present from birth; thus, its pathogenic effects are presumed to begin early in development, preceding overt neuropsychiatric symptoms. Its broad psychiatric effects reflect pleiotropic effects across neuropsychiatric phenotypes.

Plasma-based metabolomic studies of 22qDS have revealed a shift from oxidative phosphorylation to glycolysis driven by haploinsufficiency of the mitochondrial citrate transporter *SLC25A1*, as evidenced by elevated lactate/pyruvate ratios and increased levels of 2-hydroxyglutaric acid (2-HG).[4] Another plasma-based study highlighted lipid and amino acid abnormalities, including reduced taurine and increased acylcarnitines.[5] Recent investigations using dried blood spots have indicated that increased proline, attributed to *PRODH* haploinsufficiency, and decreased tyrosine, presumably related to *COMT* haploinsufficiency, are key markers that differentiate individuals with 22qDS from healthy controls (HCs).[6,7] In 22qDS mouse models, reduced palmitoyltransferase activity and changes in sphingolipid metabolism have been reported, along with alterations in ceramide phosphoethanolamines, sphingomyelin, and carnitines in the frontal cortex and hippocampus.[8] These metabolic profiles are assumed to contribute to the neurodevelopmental difficulties and psychiatric risk in 22qDS.[4,7,8]

Previous 22qDS metabolomic studies have focused on blood samples, ignoring urinary metabolites. However, as patients with 22qDS are often susceptible to anxiety,[3] they refuse to participate in blood collections, making urine sampling a better, noninvasive alternative for such patients. Importantly, blood is under tight homeostatic regulation that maintains metabolite levels within narrow physiological ranges, whereas urine contains a broad spectrum of endogenous and exogenous compounds including dietary components, pharmaceuticals, and microbial by-products.[9] Additionally, blood reflects a momentary metabolic state, and its homeostatic regulation may mask transient or low-abundance compounds, whereas urine represents a cumulative output over time, which facilitates metabolite detection.[10] Finally, the urinary metabolome is predominantly hydrophilic, whereas the serum is notably enriched in lipids.[9] Thus, the urinary metabolome is expected to have a profile distinct from that of the blood-derived metabolome.

Here, we report a pilot study comparing the urinary metabolomic profiles of 10 individuals with 22qDS with that of 10 age- and sex-matched HCs. Metabolites were analyzed using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and liquid chromatography time-of-flight mass spectrometry (LC-TOFMS). Our results indicate a metabolic signature of 22qDS that potentially reflects systemic and microbial alterations related to its neuropsychiatric manifestations.

METHODS

Participants

Ten individuals with 22qDS (three men and seven women) and 10 age- and sex-matched HC (three men and seven women) participated in this study. Eight patients with 22qDS were recruited through a website managed by our laboratory and enrolled in the 22qDS registry, which is part of the Rare Disease Data Registry of Japan (RADDAR-J; https://www.raddarj.org/). Two patients were recruited from a specialized

outpatient clinic for 22qDS at the University of Tokyo Hospital. All 22qDS diagnoses were confirmed using fluorescence in situ hybridization.

HC participants were recruited via internet referrals, message boards at several universities, and voluntary recruitment at the University of Tokyo Hospital. The participants had no self-reported personal history of psychiatric disorders. The exclusion criteria listed as follows were confirmed similarly by self-report: (1) neurological illness at any point in the lifetime; (2) traumatic brain injury with any cognitive consequences or loss of consciousness for >5 min; (3) a history of electroconvulsive therapy; (4) low estimated premorbid intelligence quotient (IQ; <70); (5) previous alcohol abuse or dependence; and (6) previous continuous substance use or substance use disorder.

This study was approved by the Ethics Committee of the University of Tokyo (approval nos. 2019059Ge-(5) [June 7, 2024], 2018015NI-(12) [April 12, 2023], and 2094-(20) [September 17, 2024]). Written informed consent was obtained from all participants after the study was explained to them in detail.

Sample collection and preparation

Urine samples were collected at the University of Tokyo Hospital between 10:00 and 15:00 without dietary restrictions. Approximately 50 mL of midstream urine was collected from each participant and promptly aliquoted into 10-mL light-protected tubes. All samples were stored at −80°C until analysis.

Metabolomics analysis

All metabolomic measurements were performed using Human Metabolome Technologies (HMT, Yamagata, Japan), following the manufacturer's protocol. Briefly, ionic metabolites were measured using CE-TOFMS, and nonionic metabolites were measured using LC-TOFMS.

CE-TOFMS

Urine samples were mixed with an internal standard solution, diluted, and ultrafiltered. The filtrates were analyzed in both cationic and anionic modes under standard HMT conditions. The mass detection range was 50–1000.

LC-TOFMS

Urine samples were mixed with isopropanol containing an internal standard, diluted, and centrifuged. Supernatants were analyzed in both positive and negative electrospray ionization modes using an octadecylsilyl column. A mass detection range of m/z 100–1700 was used.

Data processing

MasterHands v2.19.0.2 was used to extract peaks with signal-to-noise ratio (S/N) ≥ 3 and to obtain m/z, peak area, and migration (CE) or retention (LC) times. Peak areas were normalized to creatinine (cationic mode) or both creatinine and an internal standard (anionic mode); adduct/fragment ions were excluded where possible. Peaks were aligned across samples based on m/z and migration/retention times, and putative metabolite identities were assigned by matching to the HMT library (±0.5 min for migration; ±0.15 min for retention; ±10 ppm for CE-TOFMS; and ±25 ppm for LC-TOFMS). When multiple candidates remained, suffixes were appended to distinguish between them.

Statistical analysis

All statistical analyses were performed using R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Although quality control based on missingness is recommended, there is no consensus on optimal thresholds for metabolomics.[11] To avoid disregarding metabolites that appeared predominantly in one group and which could serve as key discriminatory features, we applied the modified 80% rule, retaining metabolites detected in ≥80% of the samples in either group.[12] The remaining missing values were imputed with half the minimum detected value.[13]

Creatinine-normalized peak areas were standardized and analyzed using principal component analysis (PCA), and potential outliers were detected by visual inspection of the PC1–PC2 score plot, considering that the score for any component exceeded three standard deviations. Factor loadings were expressed as Pearson's correlation coefficients (r) between standardized metabolite values and PC scores.

Between-group differences were assessed using Wilcoxon rank-sum tests, and p-values were adjusted using the Benjamini–Hochberg false-discovery-rate (FDR) procedure. Given the small sample size and limited power, we applied an FDR threshold of $q < 0.20$, as in previous studies.[14–16] Among the metabolites that met this criterion, only those with fold changes (FCs) of >1.5 or FC < 0.67 were retained as final candidate markers, following prior urinary metabolomics studies.[17,18] The smaller shifts are prone to false positives and are rarely biologically meaningful.[19,20] Sensitivity analysis was performed by removing the PCA-defined outliers.

RESULTS

Demographic characteristics of the participants

The demographic and clinical characteristics of the participants are summarized in Table 1. Participants with 22qDS were aged 13–28 years, and no significant differences were observed between the 22qDS and HC groups in terms of age, sex, or urinary creatinine concentration. Several neuropsychiatric comorbidities were observed

in the 22qDS group, including intellectual disability (60%) and anxiety disorders (30%) (Table 1).

Metabolite detection

Metabolomic profiling of 20 urine samples (10 22qDS and 10 HC) was performed using CE-TOFMS and LC-TOFMS. A total of 473 peaks were annotated with candidate compounds based on *m/z* and migration/retention time, as referenced against the HMT metabolite library. Among these, 371 peaks were detected by CE-TOFMS (193 in cation mode and 178 in anion mode) and 102 peaks by LC-TOFMS (70 in positive mode and 32 in negative mode). After filtering for missing values using the modified 80% rule, 231 metabolites were retained for primary and 223 for sensitivity analysis.

Urine metabolomic profiling

PCA was performed on all 20 participants to examine the overall data structure and identify outliers. The first two principal components (PC1 and PC2) explained a substantial portion of the total variance, with PC1 accounting for 28.0% and PC2 for 10.1%, respectively.

The PC1–PC2 score plot indicated group separation between patients with 22qDS and HC along PC2 (Figure 1). The median PC2 score was -2.87 in 22qDS and 2.45 in HC, with a significant group difference confirmed by the Wilcoxon rank-sum test ($p = 0.0091$). Metabolites with high loadings on PC2 included xanthosine ($r = -0.72$), 3-indoxylsulfuric acid (3-IS) ($r = -0.66$), 6-hydroxymelatonin ($r = -0.65$), carboxymethyllysine ($r = -0.64$), and citrulline ($r = 0.66$). Several amino acids also exhibited moderate positive correlations with PC2 ($r = 0.40$ to $r = 0.65$).

One HC participant exhibited a markedly low PC1 score (−28.6), corresponding to a *Z*-score of −3.56, which exceeds the typical threshold for outliers ($|Z| > 3$). This participant showed abnormally high urinary excretion of amino acids, and penicillamine was exclusively detected.

TABLE 1 Demographic and clinical characteristics of participants with 22q11.2 deletion syndrome and healthy controls.

Characteristics	22q11.2DS	HC	*p*-Value
Number of participants	10	10	
Male/female	3/7	3/7	1[a]
Age (years)	19.6 (4.0)	20.1 (5.7)	0.823[b]
Creatinine (mg/dL)	69.6 (32.0)	74.9 (39.0)	0.747[b]
Neuropsychiatric comorbidities			
Intellectual disability	6	N/A	
Autism spectrum disorder	1	N/A	
Anxiety disorders	3	N/A	
Obsessive-compulsive disorder	1	N/A	
Epilepsy	1	N/A	

Note: Values are shown as means (standard deviations) or counts. All comorbidities were observed only in the 22q11.2DS group.

Abbreviation: HC, healthy control.

[a]Fisher's exact test for the male/female ratio in each group.

[b]Welch's two-sample *t*-test for age and creatinine comparisons between the groups.

Identification of altered metabolites in 22qDS

Wilcoxon rank-sum tests were conducted on metabolites filtered by missingness to assess metabolic differences between the 22qDS and HC groups. To assess the influence of outliers, a sensitivity analysis was conducted after excluding one HC with an extreme PC1 score.

In our primary analysis, 11 metabolites met the significance threshold (FDR $q < 0.20$). Of these, one metabolite did not meet the FC criterion (FC > 1.5 or FC < 0.67) and was excluded, leaving 10 metabolites as final candidate markers in this study (Figure 2; Supporting Information S2: Table 1). In the sensitivity analysis, 18 metabolites satisfied the FDR threshold, of which five were excluded based on the FC criterion, resulting in 13 candidate metabolites (Supporting Information S1: Figure 1; Supporting Information S3: Table 2). All metabolites identified in the primary analysis were retained in the sensitivity analysis.

DISCUSSION

In this exploratory study, we present the first urinary metabolomic profile of individuals with 22qDS and reveal distinct signatures such as elevated 2-HG and gut microbiota-derived uremic toxins. These findings support the potential of a noninvasive urine-based screening approach.

One HC participant was identified as an outlier (PC1 *Z*-score < −3), whose aminoaciduria, with the exclusive detection of penicillamine, indicated drug-induced Fanconi syndrome. Nevertheless, the sensitivity analysis that resulted in the exclusion of this participant did not alter the primary results but confirmed their robustness. In addition, one participant with 22qDS bore another chromosomal disorder (for privacy reasons, the disorder is not disclosed), but their PCA scores clustered with those of the other participants with 22qDS (within the 95% confidence ellipse in the PC1–PC2 plot). Thus, these participants were included in the analysis.

Urinary 2-HG levels increased, and lysine levels decreased, replicating the findings of previous blood-based studies on 22qDS.[4,7] Proline, previously reported to be elevated in the blood owing to *PRODH* haploinsufficiency,[6,7] was excluded from the primary analysis because of high missingness. However, a post hoc test after outlier removal showed a 2.1-fold increase (Wilcoxon test, $p = 0.022$).

A 1.63-fold increase in urinary 2-HG levels indicates mitochondrial dysfunction. A previous study on 22qDS reported a 1.42-fold increase in plasma 2-HG levels, which was attributed to haploinsufficiency of *SLC25A1*, encoding a mitochondrial citrate

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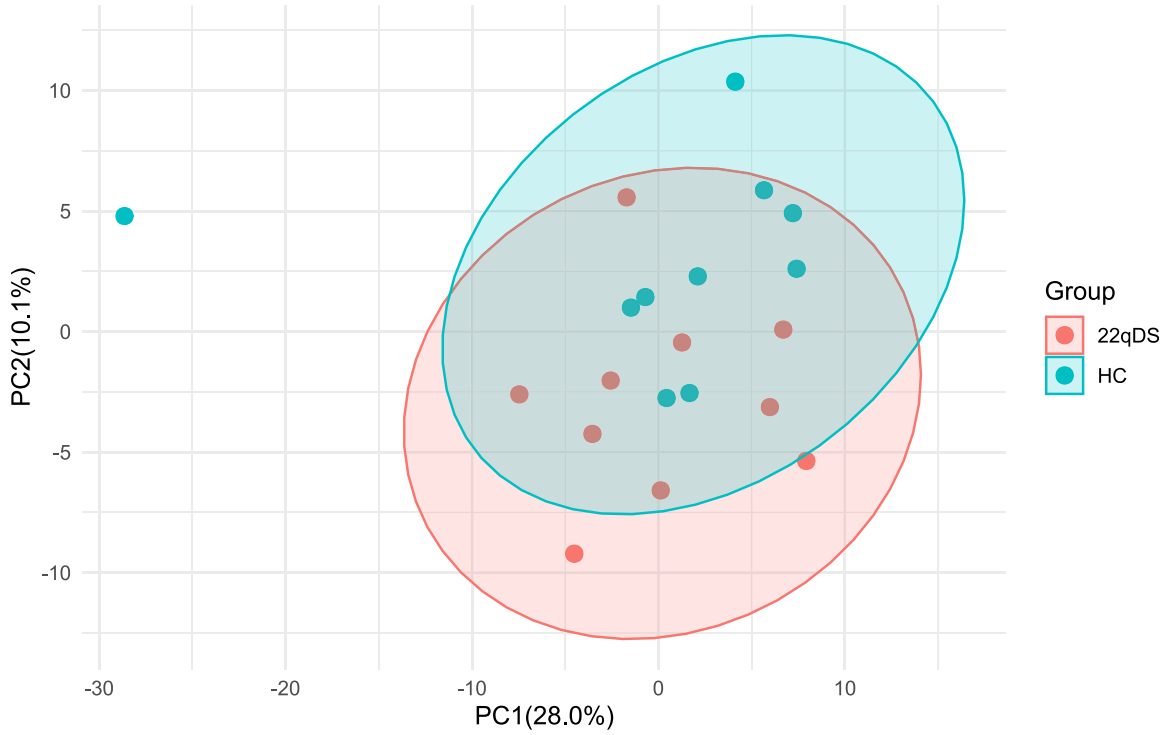

FIGURE 1 Principal component analysis of urinary metabolites in 22q11.2 deletion syndrome and healthy controls. Principal component analysis was performed on 231 metabolites after filtering and imputation. The plot indicates the first two principal components (PC1 and PC2), which explain 28.0% and 10.1% of the total variance, respectively. Each point represents an individual participant. Ellipses represent 95% confidence intervals for each group (22qDS and healthy controls [HCs]).

transporter.[4] Postmortem brain tissue-[21] and stem cell-based studies[22] on schizophrenia have similarly reported impaired mitochondrial functions. Mitochondrial dysfunction-induced oxidative stress has been consistently observed in schizophrenia.[23]

Importantly, our findings reveal a previously unrecognized increase in gut microbiota-derived uremic toxins in 22qDS urine, suggesting an underlying microbial dysbiosis and indicating the potential utility of microbiota-modulating interventions, such as probiotics and fecal microbiota transplantation. *p*-Cresol sulfate, *p*-cresol glucuronide, and trimethylamine-*N*-oxide (TMAO) are uremic toxins,[24,25] which have not been previously reported in 22qDS metabolomic studies. These findings are consistent with the high prevalence of functional gastrointestinal disorders (91%) in 22qDS.[26] They are further supported by a recent study using 22qDS model mice, which identified marked microbiome alterations in the ileum, a site of active neuroimmune signaling, although the colonic composition remained unaffected.[27] The detection of these microbiota-derived metabolites in urine, albeit not reported in blood-based studies on 22qDS, is attributable to blood homeostatic clearance mechanisms and the excretory nature of urine, which facilitates the accumulation of toxic microbial metabolites.[9]

Elevated urinary levels of *p*-cresol conjugates in 22qDS may suggest *Clostridium*-driven gut dysbiosis, which can perturb dopamine metabolism and induce oxidative stress and neuroinflammation. *p*-Cresol is reported to be produced by the bacterial catabolism of tyrosine and phenylalanine, particularly by *Clostridium* species,[28,29] and excreted mainly as *p*-cresol sulfate with minor amounts of *p*-cresol glucuronide.[30] Elevated urinary *p*-cresol levels have been consistently observed in ASD and are correlated with symptom severity.[29,30] Mechanistically, *p*-cresol disrupts catecholamine metabolism, promotes oxidative stress, and triggers neuroinflammation.[31,32] In ASD, *p*-cresol inhibits dopamine β-hydroxylase, diverting dopamine metabolism from noradrenaline toward homovanillic acid (HVA).[33] Despite *COMT* haploinsufficiency, we observed elevated urinary levels of HVA in patients with 22qDS, possibly due to a *p*-cresol-driven metabolic shift. However, vanillylmandelic acid, a major metabolite of adrenaline and noradrenaline, did not differ between groups in our dataset (FC = 1.06 in primary analysis; 1.13 in sensitive analysis). Tetralogy of Fallot, a common cyanotic congenital heart disease in 22qDS, can cause hypoxemia and compensatory increases in endogenous catecholamine secretion.[34,35] Catecholamine dynamics in 22qDS is presumably influenced by multiple factors rather than a single pathway.

In addition, TMAO was elevated, 3-IS showed a relatively strong loading on PC2 ($r = -0.66$), and citrulline was decreased in 22qDS, indicating the presence of microbial dysbiosis and intestinal dysfunction. TMAO, derived from dietary-choline metabolism, increases the risk of cardiovascular events via oxidative stress-induced endothelial dysfunction.[25,36] Growing evidence links elevated TMAO to neuropsychiatric disorders such as ASD, depression, and schizophrenia.[37]

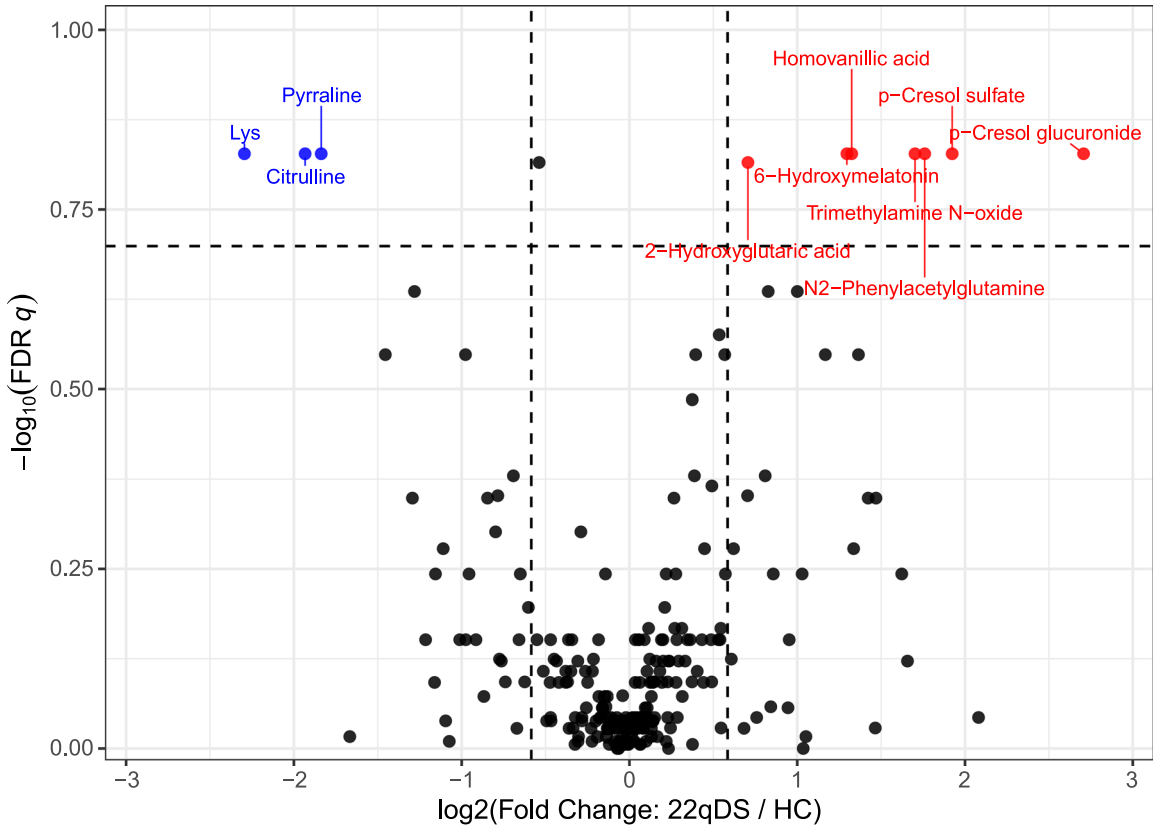

FIGURE 2 Volcano plot of differential urinary metabolites between 22q11.2 deletion syndrome and healthy controls. Volcano plot of differential expression results comparing urinary metabolite levels between individuals with 22q11.2 deletion syndrome (22qDS) and healthy controls (HCs). The *x*-axis shows $\log_2$(fold change) for 22qDS/HC, and the *y*-axis shows $-\log_{10}$(false-discovery-rate [FDR] *q*-value). Vertical dashed lines indicate fold-change thresholds of 1.5 and 0.67, and the horizontal dashed line represents the FDR threshold ($q = 0.20$). The 10 most significant metabolites are labeled; metabolites increased and decreased in 22qDS are shown in red and blue, respectively.

In ASD, elevated TMAO has been reported in both plasma and urine, although urinary levels decline in severe cases.[38,39] 3-IS, a tryptophan-derived gut microbial metabolite, causes blood–brain barrier disruption via aryl hydrocarbon signaling and is associated with anxiety and cognitive dysfunction.[40–42] Citrulline is produced by enterocytes, and its low plasma levels indicate intestinal dysfunction.[43] Similarly, we observed decreased urinary citrulline levels, a change also reported in ASD urine, which we attributed to the urea cycle and neurotransmitter dysregulation.[44]

Unexpectedly, a substantial proportion of the altered metabolites in our 22qDS study showed directionally consistent changes with those reported in patients with ASD. Citrulline,[44] lysine,[44] HVA,[31] *p*-cresol sulfate,[38] and *p*-cresol glucuronide[30] aligned with previous ASD urine findings. Pyrraline decreased, whereas TMAO increased, in the plasma of patients with ASD.[45] By contrast, phenylacetylglutamine, a bacterial metabolite of phenylalanine, increased in our 22qDS group but has been reported to decrease in ASD.[46] This contrast may reflect inhibition of phenylalanine hydroxylase resulting from catecholamine accumulation under *COMT* haploinsufficiency in 22qDS.[7] These overlaps could potentially be explained by mechanisms related to the microbiota–gut–brain axis reported in ASD, including microbial dysbiosis, increased gut permeability, and immune-mediated neuroinflammation.

The present findings suggest that focusing on urinary metabolites may be useful for developing noninvasive screening approaches in 22qDS. Where the candidate metabolites are associated with neuropsychiatric symptoms such as anxiety, noninvasive urine sampling could serve as a practical means of monitoring biological factors underlying these symptoms.

This study had several limitations. First, the small sample size limited the statistical power; therefore, a lenient significance threshold (FDR $q < 0.20$) had to be adopted. Furthermore, the associations between individual metabolite levels and psychiatric diagnoses or symptom severity in the 22qDS group could not be evaluated because of the limited power. Second, although some participants had neuropsychiatric comorbidities, none were diagnosed with a psychotic disorder at the time of assessment, and, as far as we observed, none had exhibited psychotic symptoms. Thus, the present study reflected the metabolic features of a high-risk population for psychosis rather than those of manifest psychosis. Third, although several microbiota-related metabolites have been identified, their mechanistic links to the 22q11.2 deletion remain unclear. Finally, potential confounding factors such as diet, medication use, and renal function were not controlled for, and the analysis was limited to urinary metabolites.

In summary, our work identified several microbiota-related metabolites in individuals with 22qDS, suggesting that gut microbial dysbiosis is a potential 22qDS metabolic feature. Elevated 2-HG and reduced lysine levels previously reported in blood-based studies were also detected in urine, supporting that mitochondrial dysfunction contributes to systemic and neuropsychiatric vulnerability in 22qDS. Several of our findings were concordant with those reported in ASD, indicating that the microbiota–gut–brain axis dysfunction implicated in ASD may also contribute to 22qDS. Given that 22qDS represents one of the strongest genetic risk factors for psychosis, these findings may offer insights into the systemic and microbial contributions to early pathophysiological changes underlying psychosis vulnerability. Although the clinical implications remain to be established, our results underscore the need for larger-scale studies and mechanistic investigations in this genetically defined high-risk population.

AUTHOR CONTRIBUTIONS

Takuto Minami: Conceptualization; data curation; formal analysis; methodology; writing—original draft; writing—review and editing. **Tempei Ikegame**: Conceptualization; data curation; formal analysis; methodology; project administration; supervision; writing—review and editing. **Miho Tanaka**: Data curation; methodology; resources; writing—review and editing. **Eureka Kumagai**: Data curation; writing—review and editing. **Akiko Kanehara**: Data curation; resources; writing—review and editing. **Ryo Morishima**: Resources; writing—review and editing. **Yousuke Kumakura**: Conceptualization; resources; writing—review and editing. **Noriko Okochi**: Resources; writing—review and editing. **Junko Hamada**: Data curation; resources; writing—review and editing. **Tomoko Ogawa**: Data curation; resources; writing—review and editing. **Hidetaka Tamune**: Conceptualization; resources; supervision; writing—review and editing. **Yukiko Kano**: Conceptualization; resources; supervision; writing—review and editing. **Seiichiro Jinde**: Conceptualization; data curation; formal analysis; project administration; resources; supervision; writing—review and editing. **Kiyoto Kasai**: Conceptualization; formal analysis; funding acquisition; project administration; resources, supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to restrictions imposed by the institutional ethics committee, but may be made available upon reasonable request and with prior committee approval.

ETHICS APPROVAL STATEMENT

This study was approved by the Ethics Committee of the University of Tokyo (approval nos. 2019059Ge-(5) [June 7, 2024], 2018015NI-(12) [April 12, 2023], and 2094-(20) [September 17, 2024]).

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all participants after the study was explained to them in detail.

CLINICAL TRIAL REGISTRATION

Not applicable.

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SUPPORTING INFORMATION

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