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PILOT STUDY

Food Selectivity, Gastrointestinal Symptoms and Urine Organic Acids in Autism Spectrum Disorder: A Pilot Study

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Abstract: ***Background*:** Autism Spectrum Disorder (ASD) is characterized by numerous co-morbidities including eating problems, the most common of which is food selectivity (FS), and gastrointestinal (GI) dysfunction, which often occurs concurrently with eating problems.

***Aim*:** To investigate the relationships between food selectivity, GI symptoms and various metabolic pathways in children with ASD using parental report and quantitative urine organic acid testing.

***Methods*:** An anonymous review of the clinical charts of 68 children aged 1.6 to 11 with a diagnosis of ASD was performed. Demographic and health information from intake forms and urine organic acid test reports were analyzed; descriptive statistics and Chi square tests were conducted.

***Results*:** Parents of 60% of children reported food selectivity in their child and parents of 69% of children reported GI symptoms. 47% of parents reported both food selectivity and GI symptoms in their child. 90% of the participants were found to have at least one elevated GI fungal metabolite, and 30% or more had elevated levels of 5 different GI bacterial metabolites. No significant correlation between food selectivity and GI symptoms was identified.

***Conclusion*:** This study highlights important trends among FS, GI symptoms and select organic acid metabolites; further studies of the clinical significance of these metabolites and their effect on the behavior, sensory experiences and physical symptoms among children with ASD are suggested.

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1. INTRODUCTION

Autism Spectrum Disorder (ASD) is a clinically heterogeneous, multi-system disorder, characterized by social, communication, and behavioral impairments. Once considered a genetically-based, hardwired brain disorder, studies have begun to show that ASD is in fact a whole-body, biological condition, affected by both genetic predisposition and environmental factors [1, 2]. ASD is characterized by numerous comorbidities, including sleep problems [3], seizures and epilepsy [4], ADHD [5], obsessive-compulsive disorder [3], anxiety disorders [3], and gastrointestinal dysfunction (GID) [6]. Children with ASD also commonly experience eating and feeding problems, with many suffering from some manifestation of food selectivity (FS) or picky eating [7, 8]. Close to 70% of children with ASD are reported to be selective eaters [9], a term which may refer to frequent refusals of particular foods, limited repertoires of foods, excessive intake of a few foods such as carbohydrates, and selective intake of certain foods, such as fruits and vegetables [10].

The eating and feeding problems seen in ASD are multifactorial and include sensory [10], social [11], behavioral [8, 9], physiological [12], cognitive [13] and medical [6] origins. Excessive adherence to routines and rituals and resistance to change, hypo or hyper-reactivity to sensory input, or unusual interest in sensory aspects of the environment and repetitive and restricted behaviors and interests (RRBI) are all commonly believed to contribute to FS [8]. According to Ledford and Gast (2006), FS is frequently described by type and/or texture, however, selectivity by presentation/appearance, taste, color, smell and temperature is also common. Children with ASD often display insistence on specific methods of food preparation, food types, and mealtime rules [14, 15], as is characteristic of higher order repetitive behaviors [16-18]. In addition, many children with ASD

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who are food selective often exhibit a preference for starches, snack foods, and processed foods and display a lack of willingness to eat fruits, vegetables, and proteins. In one study of 30 children with ASD, vegetables were the most commonly rejected food based on parental report; increased FS was also found to be correlated with more problem behaviors [19].

Although it is a frequent target for intervention due to concern over dietary balance, the nature of the relationship of FS to nutritional status is still unclear. Research has shown that children with ASD consume less calories, including less protein and more carbohydrates than typically developing children and have disturbed eating habits [10, 20]. However, studies on the impact of these eating problems on intake of micronutrients have yielded mixed results; for example, low levels of vitamins A and K, as well as higher levels of vitamin B6 and E have been noted, as have low intake of calcium, fiber, iron, and vitamins E and D [21].

Considering the multifactorial nature of FS, there is increasing evidence that for many children with ASD, its origin may also be organic [12]. Children with ASD have been found to have increased incidence of functional and metabolic disturbances such as an altered gut microbiome [22], higher levels of oxidative stress [1, 23-25], altered methylation and sulfur metabolism, and changes in levels of amino acids, neurotransmitters, vitamin and mineral markers [26]; alterations in cellular and neuronal development, and abnormal patterns of certain proteins/peptides, levels of neurotransmitters, hormones and markers related to an upregulated immune response have also been identified [27].

Recent literature shows that the prevalence of gastrointestinal (GI) symptoms in children with ASD ranges from 9-70% and higher [6]; children with ASD also have higher rates of GID than typically developing children [28]. A recent meta-analysis of 15 studies found that children with ASD have higher rates of diarrhea, constipation and abdominal pain than comparison groups; greater incidence of megarectum, resulting from muscle dysfunction or fecal impaction, has also been found among children with ASD, compared to controls [29]. In addition, in children with ASD, abnormalities of the GI tissue and increased intestinal permeability have been noted [30, 31] as has damage to the tight intercellular junctions of gut mucosa [32].

Furthermore, pathogenic bacterial strains such as *Clostridia* tetani, known to cause illness and produce neurotoxins that may be absorbed from the GI tract, and some strains of candida, have been identified in greater numbers among autistic children [33-36]. Lower levels of beneficial bacterial strains of *Bifidobacteria* have also been reported [36]. Research has shown that yeast and pathogenic bacterial overgrowth, a common occurrence after the use of oral antibiotics [37, 38] can also, under certain metabolic conditions become a pathogen, secreting toxins that can damage the central nervous system [39].

ASD severity and unusual sleep, oppositional behavior and rigid-compulsive behaviors have all been found to be significantly associated with GI problems among children with ASD [40-42]. In addition, symptoms such as hostility, slurred speech and ataxia have been associated with alterations in the GI microbiome [34]. Treatment with antibiotics and antifungals has been linked with marked decreases in such symptoms [1-3] and the use of antifungal medications such as Nystatin, has also been shown to lead to an improvement in ASD symptoms in some studies [43].

Indeed, the presence of GI imbalance is now known to affect the health of the brain, with studies on the "gut-brain connection" increasingly reported in the literature [4, 5]. Mechanisms involving the peripheral and central nervous systems, as well as behavior and immunological abnormalities in the GI tract have been proposed to influence ASD symptoms [44]. Although a number of causal and therapeutic hypotheses involve the GI tract, the presence of a clear GI pathophysiology specific to ASD has yet to be identified, yet elevated risk for GID in this population remains a critical clinical issue [28].

A variety of diagnostic tests including urinary organic acids can be used to assess the presence of problems with GI function, cellular energy production, mitochondrial metabolism, neurotransmitter metabolism, and vitamin status [45]. Urinary testing offers benefits including early diagnosis of metabolic disorders and neurological disease and it is simple, sensitive and non-invasive [46, 47]. Accumulations of certain metabolites, such as organic acids in the urine, can shed light on the function of numerous biochemical pathways and indicate the presence of metabolic dysfunction, nutrient insufficiencies, microbial overgrowth and more [48]. In a 2011 study, significant differences were found between the urine organic acids of 35 children with ASD and 36 neurotypical children, indicating the potential utility of organic acid testing in assessing nutritional and biochemical abnormalities in these children, and researchers have begun to explore their potential use as ASD biomarkers [6].

2. STUDY RATIONALE

Research has highlighted the occurrence of altered metabolic pathways in ASD as well as co-morbidities such as GID, which has been found to occur concurrently with eating problems such as FS [12]. Though the unique patterns of organic acids in the urine of children with ASD have been studied, to our knowledge, the relationship between these metabolites, FS and GI symptoms has yet to be explored. In a recent review, eating and feeding problems and GID were found to be more prevalent among ASD groups than non-ASD groups, with FS found to be the most common type of eating problem among children with ASD [12, 49]. Identifying biomarkers of ASD and their relationship with eating patterns may not only play an important role in understanding the etiology of this disorder, but may also contribute to the development of interventions that might improve the health and well-being of children with ASD. Therefore, the goal of this study was to explore the relationships between FS, GI symptoms and urine organic acids in children with ASD.

3. PROCEDURE

Ethical approval was received from the University Ethical Board. The health records of children whose parents sought a nutritional consultation for the purpose of seeking to optimize their child's nutrition were anonymously re-

viewed for this study. Research data included demographic and health information from intake forms completed prior to first consultation and the urine organic acid test reports from the clinical charts of children whose families elected to perform this testing. Urine testing was performed using an at home collection and shipment via courier to a medical laboratory and the same laboratory was used for all testing.

4. METHODS

An anonymous review of the clinical charts of 68 children with a diagnosis of Autism, ASD, and PDD-NOS performed. Participant ages ranged from 1.6-11 (mean age = 3.9, SD = 1.4) years (59 males and 9 females). All children were diagnosed by a developmental physician (pediatrician, neurologist) or a psychologist or psychiatrist, and met the Diagnostic and Statistical Manual of Mental Disorders (Fourth edition, Text revision) criteria for ASD. Children with typical development or with any delay other than ASD related to genetic disorders and severe medical problems were not included in this study.

5. TOOLS

1. Quantitative urine organic acids test, including the following metabolites/organic acids: citramalic, 5-hydroxymethyl 2-furoic, 3-oxoglutaric, furan-2,5-dicarboxylic, furancarbonylglycine, tartaric, arabinose, carboxycitric, tricarballyic, 2-hydroxyphenylacetic, 4-hydroxyphenylacetic, 4-hydroxybenzoic, 4-hydroxyhippuric, hippuric, 3-indoleacetic, succinic, 3-(3-hydroxyphenyl)-3-hydroxypropionic (HPHPA), 3,5-dihydroxyphenylpropionoic acid (DHPPA), glyceric, oxalic, lactic, pyruvic, 2-hydroxybutyric, fumaric, malic, 2-oxoglutaric, aconitic, citric, homovanillic (HVA), vanillylmandelic (VMA), HVA to VMA ratio (HVA/VMA), 5-hydroxyindoleacetic (5-HIAA), quinolinic, kynurenic, quinolinic-5-hydroxyindolacetic acid (HIAA) ratio, uracil, thymine, 3-hydroxybutyric, acetoacetic, 4-hydroxybutyric, methysuccinic, adipic, ethylmalonic, suberic, sebacic, methylmalonic, pyridoxic, pantothenic, glutaric acid, methylcitric, pyroglutamic, orotic, 2-hydroxyhippuric. All testing was performed at the same laboratory. Organic acids found to be elevated in 25% or more of the participants are addressed in the results and discussion.

2. An initial intake form and initial consultation which includes general questions regarding GI symptoms (presence of abnormal symptoms such as diarrhea, constipation, very soft stools, visible undigested food in stool) and questions regarding eating problems. A child was considered to be food selective if the parents reported several of the following: over-selectivity, aversions to specific textures, colors, smells, and temperatures or rigidity with respect to brands of foods, restricted intake to certain food groups such as high protein, high starch or those with certain sensory aspects such as crunchy or sweet.

6. DATA ANALYSIS

Descriptive statistics were used to illustrate the percentage of participants whose results were above the normal range for each metabolite. Metabolites for which greater than 25% of the participants had elevated levels are reported in this study. Chi-squared tests were used to assess the correlations between elevated metabolites and parental report of FS and GI symptoms and a post hoc Bonferroni correction using a revised p value of 0.01 was performed in order to correct for multiple comparisons.

7. RESULTS

7.1. Organic Acid Metabolites

Of the 53 metabolites assessed, all 68 participants had at least one elevated metabolite, and up to 24 elevated metabolites. The mean number of elevated metabolites was 9.5 (SD = 5). 25% or more of the children had 15 elevated metabolites and between 10-24% of the population had 20 elevated metabolites. The remaining 18 metabolites were elevated in under 10% of the study population (Table **1**).

Table 1. Number of children with elevated levels of organic acids.

Organic Acid	Number of Children with Elevated Levels
Arabinose	55 (80.9)
Kynurenic	18 (58.1)
4-Hydroxyhippuric	18 (48.6)
3-oxoglutaric	30 (44.1)
Oxalic	29 (43.3)
Ethylmalonic acid	22 (38.6)
Pyruvic	25 (37.3)
4-Hydroxybenzoic	17 (36.2)
HPHPA	21 (35.0)
Citric	22 (32.4)
Hippuric	12 (31.6)
Quinolinic	14 (29.8)
VMA	18 (28.1)
Succinic	15 (27.3)
Suberic acid	18 (26.9)
Methylmalonic acid	16 (23.9)
4-Hydroxyphenylacetic	16 (23.5)
Quinolinic-5HIAA ratio	11 (23.4)
Uracil	15 (22.4)
Glutaric acid	14 (20.9)
Carboxycitric	14 (20.6)
Pantothenic acid	11 (18.6)
2-Hydroxyhippuric	12 (17.9)

Table 1. Contd…

Citramalic	12 (17.6)
Aconitic	6 (17.6)
Pyridoxic acid	11 (16.7)
Methysuccinic	5 (15.2)
Orotic	10 (14.9)
Furan-25-dicarboxylic	10 (14.7)
Pyroglutamic	5 (14.7)
Adipic	9 (13.4)
5-Hydroxymethyl-2-furoic	9 (13.2)
4-Hydroxybutyric	7 (10.8)
5-Hydroxyindoleacetic	7 (10.4)
Acetoacetic	6 (10.3)
3-Indoleacetic	4 (9.8)
2-Hydroxybutyric	3 (9.1)
HVA	6 (9)
Furancarbonylglycine	6 (8.8)
Tartaric	6 (8.8)
Glyceric	5 (8.8)
DHPPA	3 (8.6)
Lactic	4 (6)
HVA/VMA	3 (6)
3Hydroxybutyric	4 (6)
2-oxoglutaric	4 (5.9)
Thymine	3 (5.3)
2-Hydroxyphenylacetic	2 (4.7)
Fumaric	3 (4.5)
Tricarballyic	1 (3.8)
Methylcitric	2 (3.6)
Sebacic	1 (3)
Malic	1 (2)

(Number as percentage is listed in parentheses).

The following 15 metabolites were elevated among 25% of more of the participants: arabinose, kynurenic acid, 4-hydroxyhippuric acid, 3-oxoglutaric acid, oxalic acid, ethylmalonic acid, pyruvic acid, 4-hydroxybenzoic acid, HPHPA, citric acid, hippuric acid, quinolinic acid, VMA, succinic acid and suberic acid.

7.2. FS and GI Symptoms

Parents of 60% of children reported FS in their child and parents of 69% of children reported GI symptoms in their child. Seventy-eight percent of parents who reported FS in their child also reported GI symptoms; in contrast, 59% of parents who did not report FS reported GI symptoms. Of the 68 children assessed, 24% reported both FS and GI symptoms. No statistically significant correlation was found between parent-reported FS and GI symptoms. Ninety percent of the participants studied were found to have at least one elevated GI fungal metabolite, and 30% or more had elevated levels of 5 different GI bacterial metabolites.

Of the 15 metabolites which were elevated among 25% or more of the participants, 12 of them were also elevated among 40% or more of children reported to have FS and 8 of them were elevated among 40% or more of children reported to have GI symptoms. Six of those metabolites were elevated among 40% or more of children reported to have both FS and GI symptoms (Table **2**).

7.3. FS and GI Correlations

Correlations between 5 organic acids and FS were initially identified, which included vanillylmandelic acid (VMA), suberic acid, 3-oxoglutaric acid, arabinose and oxalates. However, after a post-hoc Bonferroni correction was carried out, the correlations did not remain significant at the new p value of 0.01. Likewise, the correlation identified between an organic acid, carboxycitric acid and gastrointestinal symptoms did not remain significant at the p value of 0.01.

8. DISCUSSION

Food selectivity is a common co-morbidity of children with ASD [7, 8] and has previously been shown to be related to RRBIs, with origins in sensory, behavioral and social impairments. However, this study and previous studies indicate that for many children, eating and feeding problems may also be organic, with many cases co-occurring with GID.

In this pilot study, which explored the relationships between parent-reported FS, GI symptoms and urine organic acids, FS was reported by 60% of parents and GI symptoms were reported by 69%. Furthermore, 47% of parents reported both FS and GI symptoms in their children. Though no statistically significant differences were found between the FS group and the no FS group in terms of GI symptoms, the parents of children with FS reported a higher rate of these problems, suggesting that FS may be related to GI symptoms in some children [49].

The descriptive statistics of the urinary metabolites reveal important trends relating food sensitivity and metabolic imbalance. Of the 53 metabolites tested, 15 were elevated in 25% or more of the children studied and 6 of the metabolites were associated with gastrointestinal bacteria and fungi (arabinose, 3-oxoglutaric, 4-hydroxyhippuric, 4-hydroxybenzoic, HPHPA, hippuric acids) [38, 45, 50, 51]. Ninety percent of the participants were found to have at least one elevated fungal metabolite, and 30% or more had elevated levels of 5 different bacterial metabolites. One of these, arabinose was elevated in the greatest number of children (80.9%), and was also identified in 73% among those who reported FS, 76.6% of those who reported GI symptoms and 72% of those who reported both FS and GI symptoms.

Table 2. Organic acids elevated among 25% or more participants & occurrence with FS & GI.

Metabolites	Category	FS%	GI%	FS & GI%
Arabinose	Yeast & Fungal	73.2	76.6	71.9
3-oxoglutaric	Yeast & Fungal	34.1	40.4	37.5
4-hydroxyhippuric	Bacterial	57.7	50	57.9
4-hydroxybenzoic	Bacterial	37.5	38.2	37.5
HPHPA	Bacterial	37.8	35	39.3
Hippuric acid	Bacterial	30.8	37	31.6
VMA	Neurotransmitter	17.9	23.3	13.3
Kynurenic	Neurotransmitter	53.8	45	60
Quinolinic	Neurotransmitter	28.1	23.5	20.8
Oxalic	Oxalates	33.3	40	33.3
Ethylmalonic	Fatty acids	51.5	41	53.8
Suberic	Fatty acids	17.5	26.1	16.1
Pyruvic	Energy production/mitochondria	47.5	43.5	51.6
Citric	Energy production/mitochondria	38.5	40	40
Succinic	Energy production/mitochondria	23.5	26.3	25.9

Arabinose is a close relative of arabinitol, a yeast alcohol, and has been previously proposed as a biomarker for Candidiasis [52]. Elevated levels have been found in various clinical populations, including patients with schizophrenia, women with vulvovaginitis, in children with conduct disorders and among children with ASD [38, 53]. D-arabinitol and the D-/L-arabinitol ratio have been more widely discussed in the literature as the major characteristic metabolites of most Candida species [54] and the D-/L-arabinitol ratio specifically has been shown to be a sensitive and rapid test for invasive candidiasis [55, 56]. Arabinitol has also been found to be three times higher among autistic children compared to healthy children [57] and both probiotic supplementation [58] and treatment with anti-fungal medication have both been shown to reduce elevated levels [54].

In addition, 3-oxoglutaric acid was also elevated among 34% of children whose parents reported FS, 40.4% of those who reported GI symptoms and 37.5% among those who reported both. 3-oxoglutaric acid was previously identified in a small study in which two boys with ASD with elevated levels responded to treatment with Nystatin, an anti-fungal drug [38], indicating, like arabinose, a possible relationship with GI dysbiosis. It has been found to play a role in detoxification of ammonia in brain [59-61] and is also considered a Krebs cycle intermediate is a precursor to the excitatory neurotransmitter glutamate, which may also be decarboxylated (in the presence of vitamin B6) into the chief inhibitory neurotransmitter, GABA. GABAergic dysfunction has been implicated in anxiety, epilepsy and learning impairment, amongst other conditions. Numerous studies have identified abnormalities in GABAergic neurons and synapses in children with ASD [62] with polymorphisms in GABA receptors having been identified among children with ASD [63]. Glutamate excitotoxicity based on a much higher glutamate concentration in autistic patients than control subjects, and higher GABA and lower glutamate/GABA levels have been recorded in autistic patients, indicating possible imbalances between excitatory and inhibitory neurotransmission [64]. A recent 2016 study found sensorimotor GABA levels to be significantly reduced in children with autism compared to healthy controls which the authors noted might be predictive of abnormal tactile information processing in ASD [65].

Although the relationship between intestinal fungal overgrowth and selective eating has not been discussed in the literature, the impact of diet on the gut microbial population is widely recognized [66, 67]. To our knowledge, this is the first pilot study to identify elevation in arabinose among over 70% of children with reported FS and in 3-oxoglutaric acid among 34% of children with reported FS and further research is suggested in order to shed further light on its potential relationship with FS mechanisms, including its potential role in restriction to certain foods, GI discomfort and influence on food choices.

Four different metabolites related to bacterial overgrowth, 4-hydroxyhippuric acid, 4-hydroxybenzoic acid, HPHPA and hippuric acid, were found to be elevated in 30% or more of children with both FS and GI symptoms. Children with ASD have been repeatedly shown to have a significantly greater number of bacterial species in their stool samples, along with grossly abnormal bacterial flora [35, 68]. A growing body of research has indicated that children with ASD have lower levels of both of *Bifidobacteria* and *Lactobacillus* species [36, 68, 69] and greater numbers of GI pathogens, including high levels of bacterial taxa belonging to *Escherichia/Shigella* and *Clostridium* and *Candida* species [36, 70, 71]. In addition, studies of fecal samples of chil-

children with ASD have identified the presence of abnormal amounts of *Clostridia*, *Bacterioides* and *Desulfiovibrio*, as well as decreased amounts of *Bifidobacteria* [40]. In a study of 58 children with ASD and 39 healthy, typical controls, children with ASD were found to have much lower levels of total short chain fatty acids, less *Bifidobacteria* and higher amounts of *Lactobacilli* [36]. The same study found GI symptoms to be strongly correlated with autism severity. One of the 4 elevated bacterial metabolites, 3-(3-hydroxyphenyl)-3-hydroxyproprionic acid or HPHPA, is an abnormal metabolite of *Clostridia* and has also been found to be elevated in the urine of children with autism, compared to matched controls for age and sex. One study found the marker to decrease following treatment with Metronidazole, indicating the production of the compound by one or more anaerobic bacteria [38]. As with the fungal metabolites, the high percentages of GI symptoms and FS among children with elevated bacterial metabolites identified in this pilot warrants a deeper exploration of the relationship between these co-morbidities.

Among those with elevated levels of VMA 18% had FS and 23% had GI symptoms. VMA is a metabolite of the catecholamines epinephrine (adrenaline) and norepinephrine, released into the bloodstream in response to physical or emotional stress. Indeed, dysfunction in dopaminergic signaling has been discussed as an underlying cause of different neuropsychiatric disorders, including ASD [72, 73]. While several studies have identified significant differences in the urinary levels of HVA and VMA of autistic and healthy children [74], others have not identified abnormal levels among this population [75].

Abnormalities in the dopamine-based modulation of frontal systems have also been explored as a contributor to the development of executive dysfunction in ASD [76]. Executive cognitive function encompasses the skills required to carry out goal-directed activity, and is comprised of higher order processes related to self-regulation and shown to predict a range of life outcomes including health behaviors and academic performance [7]. As a result, the executive dysfunction seen in ASD has been suggested to influence eating behavior as the lack of ability to plan and control behavior may cause a 'locking' into a certain types of food or brands and stress surrounding food–related changes [8]. The snack foods commonly consumed by children with ASD may be extremely rewarding for them, when consumed, and place a high emotional and motivational drive upon immature executive cognitive systems, making it difficult to inhibit these highly desirable foods [9]. Such deficits in inhibitory control have been associated with characteristics of FS in previous studies, such as poorer eating behavior, and consumption of unhealthy foods [77]. However, the specific relationship between VMA and FS warrants further exploration.

Both kynurenic and quinolinic acid, both of which have tryptophan as a precursor, were found to be elevated among 25% or more participants, with kynurenic acid elevated among 53.8% of those reporting FS, 45% of those who reported GI symptoms and 60% of children with both FS and GI symptoms. The kynurenine pathway is the most tryptophan-consuming pathway and it can result in the production of numerous metabolites, including NAD, kynurenic acid, quinolinic acid and picolinic acid. Elevations in kynurenic acid have been reported to cause dysregulation of gut motility [78] and may also influence important neurophysiological and neuropathological processes and high levels have been identified in human urine in certain metabolic disorders, such as marked pyridoxine deficiency. In addition, quinolinic acid has previously been found to be elevated among children with ASD in a small study of Omani children also found increased production of the downstream metabolite, quinolinic acid, which is capable of enhancing glutamatergic neurotransmission [79] and Gevi et al found children with ASD preferentially transform tryptophan into xanthurenic acid and quinolinic acid (two catabolites of the kynurenine pathway), at the expense of kynurenic acid and especially of melatonin [80]. More research is needed to further understand potential relationships.

This study also found 2 elevated fatty acid metabolites, suberic acid and ethylmalonic acid, elevated among 25% or more of the participants. Among those reported to be food selective, 17.5% had elevated suberic acid, as did 26.1% who reported GI symptoms and 16% who reported both. Elevated ethylmalonic acid was identified among 51.5% of children with FS, 41% of participants with GI symptoms and 53.8% who reported both. The presence of extremely elevated levels of suberic acid has been found in the urine of patients with fatty acid oxidation disorders, a broad classification for genetic disorders that result from an inability of the body to produce or utilize an enzyme required to oxidize fatty acids to produce energy [81]. Among the ASD population, significant differences in suberic acid levels between children with ASD and neurotypical controls were previously reported in a 2010 study [6] and various forms of abnormal fatty acid metabolism among children with ASD have also been found in other research [82, 83]. There is also evidence that highlights the abnormalities of fatty acid and membrane phospholipid metabolism present across a range of neurodevelopmental disorders [84]. Supplementation with riboflavin (vitamin B2) and carnitine has been proposed as a treatment for elevated suberic acid, however, its relationship with FS was not previously reported and therefore specifically warrants further exploration [6].

Among those with elevated levels of oxalates**,** 33% were food selective, 40% had GI symptoms, and 33.3% reported both. A component of kidney stones as a calcium precipitate, oxalate and its acid form, oxalic acid is organic acid produced primarily from three sources: the diet, fungi [85-87], and a byproduct of human metabolism [88]. Oxalate metabolism is partially dependent on a strain of GI bacteria, *Oxalobacter formigenes*, an oxalate-degrading anaerobic bacterium present in the large intestine and the primary source for the Oxalyl-CoA decarboxylase enzyme, without which, the GI tract cannot degrade dietary oxalates. When not properly degraded, oxalates may become absorbed or excreted via the kidney. Hyper oxalemia and hyperoxaluria may be involved in the pathogenesis of ASD in some children [51, 89]. Proper breakdown of oxalates is partially dependent on a healthy microbiome [90], which many children with ASD lack; it has also been hypothesized that excess presence of oxalate may lead to a deposition of crystals in the GI tissues, which may contribute to increased intestinal permeability [91]. The specific connection between FS and high oxalate foods has yet

to be explored, though possible mechanisms may include increased food allergies and sensitivities, and subsequent FS resulting from increased intestinal permeability or "leaky gut [92-94]."

Finally, over 50% of children with elevated pyruvate, 40% with elevated citric acid and over 25% with elevated succinic acid had both FS and GI symptoms. All three of these metabolites are intermediates of the Krebs cycle, which occurs inside the mitochondria. Numerous studies have shown a large number of children with ASD with elevations in these three metabolites in blood, urine and cerebrospinal fluid, which commonly indicate dysfunction of the mitochondria [95]. Frye *et al*. reported that GI disturbances are common in individuals with mitochondrial disorders and reported to be highly prevalent in individuals with ASD and mitochondrial disease [96]. The effect of mitochondrial dysfunction on GI function can also have a direct impact on eating and feeding, affecting gut motility and manifesting as gastroesophageal reflux, slow gastric emptying with bloating and pain, intestinal dysmotility with the same symptoms, or pseudo-obstruction, as well as constipation with fullness, pain or gas. Gut dysmotility likely occurs at least in part because of autonomic dysfunction and/or bowel smooth muscle weakness [97]. Any and all of these may also potentially manifest as FS, thus further exploration and larger studies are again, suggested.

This study has several important limitations. First, the number of charts studied was moderate therefore, further research should study larger populations of children and a control group for comparison. The chart review methodology and use of general parental report relies on information that is prone to bias. Specifically, the reports of both FS and GI problems by parents is often problematic since definitions of what constitutes both terms may vary [6]. Since the current study was retrospective, it did not allow for the inclusion of a control group, nor was there the possibility of obtaining additional data regarding food selectivity, however the results serve as a bridge to future studies in which will expand upon the available data. In addition, the retrospective nature of this study did not allow the gathering of additional, important data about types and patterns of selectivity. Finally, the limited literature on the urine organic acid metabolites and relative ambiguity regarding in the origin and significance of excreted compounds limits the breadth of the discussion. All of these call for further research, which should also include control groups for comparison.

The strengths of the pilot study include the exploration of an under-studied and important topic which make a valuable contribution to the literature.

CONCLUSION

This pilot study highlights important trends among FS, GI symptoms and select organic acid metabolites. Together with altered metabolic pathways related to the GI tract, the central nervous system, and fatty acid metabolism, the results warrant exploring whether FS may also have organic origins in addition to representing a stage of RRBI. Though no significant correlations were identified between FS, GI symptoms and organic acid metabolites, in light of these interesting trends, further studies of the clinical significance of these metabolites and their effect on the behavior, sensory experiences and physical symptoms among children with ASD are suggested.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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