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ORIGINAL ARTICLE

Is urinary indolyl-3-acryloylglycine a biomarker for autism with gastrointestinal symptoms?

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Abstract

An autism spectrum disorder (ASD) diagnosis is based on clinical behaviours as there are no validated biological diagnostic tools. Indolyl-3-acryloylglycine (IAG) is a chemical produced by gut microflora and there are conflicting reports as to whether urinary levels are elevated in children with ASD compared with controls. Urinary IAG levels in morning urine samples were statistically significantly higher in children with ASD whose caregivers reported the presence of chronic gastrointestinal (GI) disturbance than children with ASD without chronic GI disturbance. Urinary IAG, however, was not statistically significantly higher in children with ASD, compared with siblings or unrelated controls without ASD.

Keywords: *Autism spectrum disorder; indolyl-3-acryloylglycine; gastrointestinal disturbance; diagnosis; urinary biomarker; subtypes*

Introduction

Autism is a complex multi-aetiological disorder which presents as a triad of deficits where an affected person will have difficulties with communication, socialisation and behaviours (Bacchelli & Maestrini 2006). In recent years, the definition and criteria for diagnosing autism has been revised and broadened to include milder and more common forms of the disorder. Autistic disorder (AD) is classified in the Diagnostic and Statistical Manual of Mental Disorders (4th edn, DSM-IV) as one of five related pervasive developmental disorders (PDDs) (American Psychiatric Association 2000). The remaining four PDDs are pervasive developmental disorder – not otherwise specified (PDD-NOS), Asperger syndrome (AS), childhood disintegrative disorder and Rett disorder. Autism spectrum disorder (ASD) is an umbrella term that is used to represent a broad heterogeneous disorder by collectively grouping AD, AS and PDD-NOS.

ASDs carry a greater risk of comorbidities. Between 6% and 10% of children have a medical condition that might have lead to AD (e.g. fragile X syndrome, tuberous sclerosis and neurofibromatosis) leaving 90% of the cases to be idiopathic (Fombonne 2005). It is reported that as many as 35% of individuals with childhood autism have epilepsy (Turk et al. 2009) and approximately 50% of individuals with an ASD have an IQ in the intellectual disability range (Charman 2008). Epidemiological studies estimate prevalence rates of 4 per 1000 for AD and 12–16 per 1000 for the broader spectrum disorder (Baird et al. 2006, Baron-Cohen et al. 2009). The Autism and Developmental Disabilities Monitoring Network of the Centers for Disease Control and Prevention released data in 2007 that found about 1 in 150 8-year-old children in multiple areas of the United States had an ASD (CDC 2009). A 2007 report commissioned by the Australian Advisory Board for ASD estimated a 1 in 160 prevalence of ASD across Australia among 6 to 12-year-old children (MacDermott et al. 2007).

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Many theories have been proposed to account for the deficits in affected individuals ranging from changes in brain structures to metabolic impairment. However, a common aberration is not consistently seen in all cases of ASD suggesting that it is a cluster of disorders with each having its own distinct pathophysiology.

Of particular interest to this study is the link between gastrointestinal (GI) dysfunction and autism. The high frequency of GI disturbance occurring in individuals with autism was first reported almost 40 years ago (Goodwin et al. 1971, Walker-Smith & Andrews 1972). Recent studies have confirmed a high prevalence of GI symptoms (abdominal pain, constipation, diarrhoea and alternating constipation and diarrhoea) and GI inflammation in children with autism (Horvath & Perman 2002a, b).

There have been various theories proposed to explain how impaired GI function may disturb neurological development and/or function resulting in autism, including absorption of opioid-like peptides that are derived from gluten and casein (i.e. the 'opioid excess theory', Reichelt et al. 1997), GI overgrowth of neurotoxic bacteria and dysfunction of secretin or its receptors (Shattock & Whiteley 2002, Molloy & Manning-Courtney 2003, Sandler et al. 2000, Horvath et al. 1999). Functional changes have also been reported in the GI tracts of children with autism including increased intestinal permeability, i.e. a 'leaky gut', and decreased digestive enzyme activity (Horvath & Perman 2002a).

Urinary indolyl-3-acryloylglycine (IAG) (Figure 1) is a regular constituent of human urine and is produced by gut microflora. Urinary IAG has been identified as being elevated in Hartnup's disease, polymorphous light dermatosis and phenylketonuria (Jepson 1978, Verhagen & Burbach 1966, Marklova et al. 1975). A study examining urinary IAG levels in children with ASD versus controls showed that the IAG:creatinine ratio was significantly higher in individuals with ASD (Bull et al. 2003). When a larger group of children with ASD were investigated and compared with matched controls, no significant difference was found (Wright et al. 2005). In addition, another study by Whiteley and Shattock (2003) found no overall association between IAG and ASD which they speculated may have been due the effects of anti-epileptic medication taken by participants on high-performance liquid chromatography (HPLC) results.

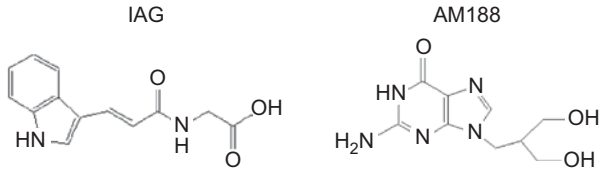

Figure 1. The structures of indolyl-3-acryloylglycine (IAG) and AM188 (IS).

It has been speculated that high levels of IAG in urine are indicative of gut dysbioisis. Shattock and Whiteley (2002) have suggested that because of its planar geometry, indolyl-3-acrylic acid (IAA), the acid precursor of IAG may disrupt membrane structures, and in turn increase the permeability of membranes. Then, excessive levels of endogenous opioid-like peptides as described by Reichelt et al. (1997) pass through the intestinal and blood–brain barrier into the brain, which increase social withdrawal and stereotypic behaviours and underpins the aetiology of ASD.

A preliminary study indicated that a link may exist between IAG and the peptide gluten (Whiteley et al. 1999). Coeliac disease is a GI disorder triggered by gluten ingestion in genetically predisposed individuals. It has been suggested that a link exists between coeliac disease and autism, at least in subgroups (Erickson et al. 2005). The gluten-free diet is widely implemented in autism and it has been theorised that it may ameliorate autistic behaviours as gluten/casein peptides have negative pharmacological effects on attention, brain maturation, social interaction and learning. However, there have only been two small randomised controlled trials and evidence for efficacy is conflicting (Millward et al. 2008).

Our study had two major aims. Firstly, to clarify whether any differences exist between ASD and control children regarding urinary IAG levels. The second aim was to determine whether within the ASD group urinary IAG levels are associated with symptoms of ongoing GI disturbance.

Methods

Participants

Samples were collected from children with ASD ($n = 57$) enrolled in the Early Intervention Research Program (EIRP) at Flinders University and Autism South Australia (Autism SA), including 45 with AD and 12 with AS. The inclusion criteria for the ASD group were that a diagnosis of AD or AS had been made by a multidisciplinary team using the Childhood Autism Rating Scale (Schopler et al. 1980) and/or the Diagnostic and Statistical Manual of Mental Disorders (4th edn, DSM-IV) (American Psychiatric Association 2000). This is a rigorous process that occurs when individuals with autism are diagnosed in South Australia (SA) and is a requirement to receive services through Autism SA, the key autism service provider in the state. Children who met the criteria for AD or AS but who presented with a comorbid diagnosis of a chromosomal abnormality,

were excluded from the study. Participants who had a primary diagnosis of AD and who also had intellectual disability as a comorbidity were not excluded. Three of the 57 children with ASD had epilepsy as a comorbidity. We also recruited 50 typically developing siblings (SIB) of the ASD cohort as well as 56 age-matched unrelated community controls (CON) without a family history of autism. The CON participants were eligible to participate if they did not have a sibling or a first cousin with an ASD. ASD was excluded in participants of both the SIB and the CON control groups by administering the Autism Spectrum Screening Questionnaire (ASSQ) (Ehlers et al. 1999). The gluten-free diet was implemented for 14 children with ASD and two siblings. All families were sent a letter describing the study and invited to express an interest in participating. Written parental consent was obtained. Two early morning urine samples (first production) were collected on non-consecutive days and stored at −80°C until analysis.

Clinical data collection

Diagnostic data were obtained from participants' EIRP or Autism SA case records. In addition self-administered surveys were mailed to caregivers. To determine whether their child experienced chronic/ongoing GI issues they were asked the following question. 'Does your child have any chronic/ongoing gastrointestinal issues i.e. bloating, diarrhoea, constipation, excessive flatulence, abdominal pain?'

Materials

HPLC-grade acetonitrile was obtained from Biolab (Adelaide, Australia). IAG was purchased from Biosynth AG (Staad, Switzerland). Creatinine and ammonium acetate were purchased from Sigma-Aldrich (Sydney, Australia). AM188 (98% assay purity) (Figure 1) was used as an internal standard (IS) (Wang et al. 2006) and was purchased from Amrad Corporation Limited (Melbourne, Victoria, Australia). Water (conductivity >18MΩ at 25°C) was purified for HPLC analysis using a Milli-RQ ultrapure Water System (Millipore, Billerica, Massachusetts, USA).

Urinary analysis

Urine samples were diluted with HPLC mobile phase (5% acetonitrile in 5 mM aqueous ammonium acetate buffer) and analysed for IAG and creatinine using LC–tandem mass spectrometry (LC-MS/MS).

The HPLC system consisted of two Shimadzu LC-10ADVP pumps, a DGU-14A degasser and SIL-HTC auto sampler (Shimadzu, Japan) kept chilled at 10°C. The separative system consisted of a HPLC Gemini column (C18, 2.0 × 50 mm, 3 µm, Phenomenex, Australia) with a matching guard column (2.0 × 4 mm, 3 µm). The HPLC system was interfaced via an electrospray ionisation (ESI) source with a triple-stage quadrupole mass spectrometer (API 3000, Applied Biosystems, Canada). Optimal declustering potential (DP), collision energy potentials (CE), collision exit potentials (CXP) and focusing potential (FP) were determined based on the relative intensities of selected product ions (Table 1) for each analyte. The needle voltage of the turbo ion spray was optimised at 4500 eV and −4500 eV for positive and negative ion mode with source temperature of 200°C, respectively. Nitrogen used as the curtain, nebuliser and collision gas is also shown in Table 1.

A gradient mobile phase was used to analyse IAG ranging from 5% acetonitrile in 5 mM ammonium (aq) acetate buffer (A) to 95% acetonitrile (B). The gradient proceeded from 0% B at time 0–1 min, a linear increase to 30% B at 1.1 min until 4 min and then a linear decrease from 30% to 0% B within 0.1 min. The total solvent flow rate was 0.2 ml min^{-1}. Negative ion pairs of *m/z* 243.1→141.9, *m/z* 238.1→149.9 and 238.1→220.0 were used to monitor IAG and the IS, respectively. Peaks corresponding to both the *cis*- and *trans*- isomers of IAG were quantified although signals were not assigned specifically to either. The total run-time was 9 min per sample and retention times of the two isomers of IAG were 4.6 and 4.9 min. In contrast, a mobile phase of 5% acetonitrile in 5 mM ammonium (aq) acetate buffer was used for creatinine analysis with IS. Positive ion pairs were monitored at *m/z* 113.9→86.1 and 113.9→72.2 (creatinine) and 240.1→152.1 (IS). The total run-time was 4 min with retention time of creatinine being 1.2 min and no solvent inference was observed.

Table 1. The optimised settings for analysis of indolyl-3-acryloylglycine (IAG), creatinine and AM188 (IS) by tandem mass spectrometry.

Analyte	MRM ions Q1→Q3 (*m/z*)	DP (eV)	CE (eV)	CXP (eV)	FP (eV)	NEB (l min^{-1})	CUR (l min^{-1})	CAD (l min^{-1})
IAG	243.1→141.9	−37	−28	−10	−200	12	8	6
AM188(IS) (Negative ion pairs)	238.1→149.9	−58	−26	−11	−230	12	8	6
	238.1→220.0	−56	−22	−18	−300	12	8	6
Creatinine	113.9→86.1	28	16	16	200	8	8	4
	113.9→72.2	34	23	13	200	8	8	4
AM188(IS) (Positive ion pairs)	240.1→152.1	64	30	14	220	8	8	4

DP, declustering potential; CE, collision energy potentials; CXP, collision exit potentials; FP, focusing potential; NEB, nebuliser; CUR, curtain.

Method validation

All samples were randomly analysed within stability parameters established during the method validation. As IAG and creatinine are endogenous metabolites, the mobile phase was used to prepare the standard curve. The validity of this method was tested by studying the matrix effect of urine compared with mobile phase. Urine gave no statistically different result. Six replicates of quality controls (QCs) analyses gave a variation of less than 6%. The calibration curve was constructed by applying a weighting of 1 y^{-2} of total peak area ratio (IAG/creatinine:IS) versus concentration of IAG/creatinine. All coefficients of correlation (r^2) of the calibration lines ($n=6$) were better than 0.995 for IAG and creatinine.

Stability tests of the analytes were performed on six replicates of QCs' concentration after (i) three freeze (−80°C)-and-thaw cycles in 3 days, (ii) keeping at 10°C for 24h in auto-sampler and (iii) stored at −80°C for a year, respectively. Concentrations of IAG and creatinine in the stored samples were compared with freshly prepared samples at the same nominal concentration. The difference between the tested samples and freshly prepared samples at three QC concentrations was less than 8%.

Statistical analysis

IAG and IAG: creatinine ratio results were normalised by natural logarithm transformation as per Wright et al. (2005). Analysis of covariance was primarily used to determine the association between either IAG or the IAG:creatinine ratio and various subgroups, controlling for any small differences in age between the subgroups by including age as a covariate. A p-value of less than 0.05 was considered statistically significant. Differences between subgroups were visually displayed using a box plot with the line across the inside of the box representing the median value, the protruding lines (whiskers) from the box go out to the variable's smallest and largest values and the small circles and stars on the graphs are outliers which extend more than 1.5 and 3 times from the edge of the box, respectively. The IAG:creatinine ratio values were age adjusted prior to plotting by using the residuals of the regression model between the IAG:creatinine ratio and subject age. Statistical analyses and tests were conducted using SPSS for Windows™ (version 17, SPSS Inc., Chicago, IL, USA, 2008) and the R statistical language.

Ethics approval

Ethics approval for this study was granted by the Human Research Ethics Committees of the University of South Australia and The Flinders University of South Australia in accordance with the Declaration of Helsinki.

Results

IAG and creatinine were measured in the urine of all participants. The sample size, age, gender distribution and category of ASD diagnosis of the participants are summarised in Table 2. Most participants supplied two morning urine samples on non-consecutive days.

Urinary creatinine concentrations in the CON group were significantly higher than all other groups when compared separately ($p<0.001$).

As per the data of Wright et al. (2005), the distribution of our IAG:creatinine ratio data was skewed to the left and therefore normalised by natural logarithm transformation. Similarly, the natural logarithms of raw IAG values were obtained. The transformed values were used in all subsequent analyses.

Across all study participants there was no statistically significant association between either urinary IAG or the IAG:creatinine ratio and participants' gender ($p=0.641$ and $p=0.724$, respectively). However, there was a statistically significant association between the IAG:creatinine ratio and the age of the participants ($r=-0.34$, $p<0.001$). This was predominantly due to a correlation between age and urinary creatinine concentration ($r=0.48$, $p<0.001$). In order to avoid confounding issues due to the small differences in age between the groups, the IAG:creatinine was adjusted for age in subsequent analyses.

The distribution of the resulting urinary IAG and IAG:creatinine ratio (age adjusted) for the ASD, SIB and CON groups is displayed in Figure 2a and 2b, respectively. An analysis of covariance including age as a covariate did not detect a statistically significant difference between the mean IAG and IAG: creatinine in the three groups ($p=0.664$ and $p=0.138$, respectively).

Table 2. Participants' demographics.

Participants	Total number of participants	Number of participants implementing gluten-free diet	Age (months) (Mean ± SD)	Gender (Male/female)	Diagnosis
ASD	57	14	82.7 ± 47.7	48/9	45 AD, 12 AS
SIB	50	2	104.0 ± 63.9	28/22	–
CON	56	0	102.0 ± 47.6	28/28	–

ASD, autism spectrum disorder; SIB, typically developing siblings; CON, unrelated community controls.

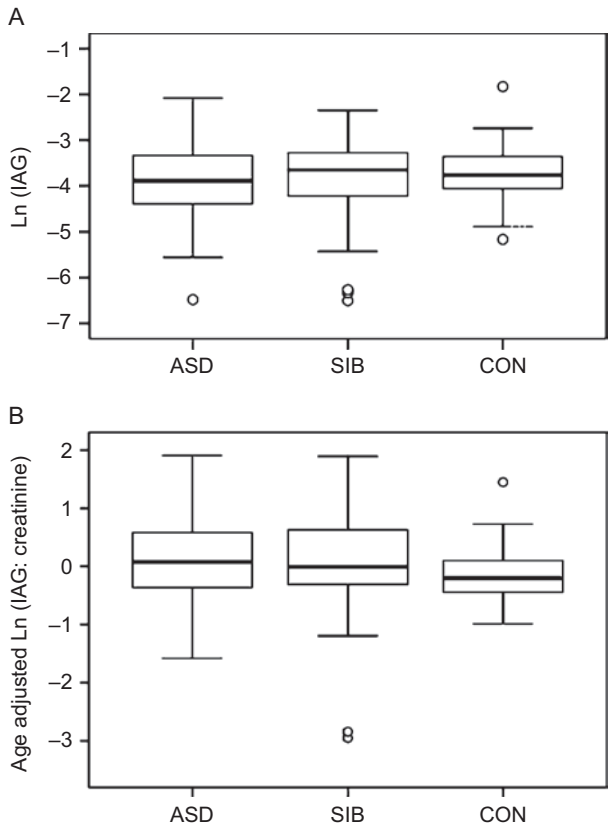

Figure 2. Box plot displaying the distribution of natural logarithm (A) indolyl-3-acryloylglycine (IAG) and (B) age-adjusted natural logarithm of IAG:creatinine in children with autism spectrum disorder (ASD), typically developing siblings (SIB) and unrelated healthy controls (CON).

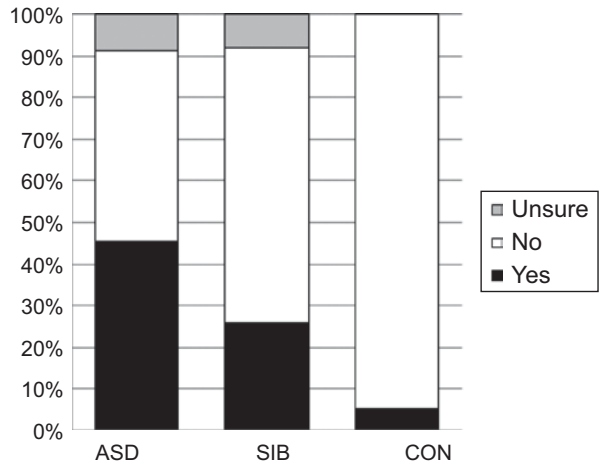

Figure 3. The proportion of participants' caregivers reporting gastrointestinal disturbance in each of the three study groups: children with autism spectrum disorder (ASD), typically developing siblings (SIB) and unrelated healthy controls (CON).

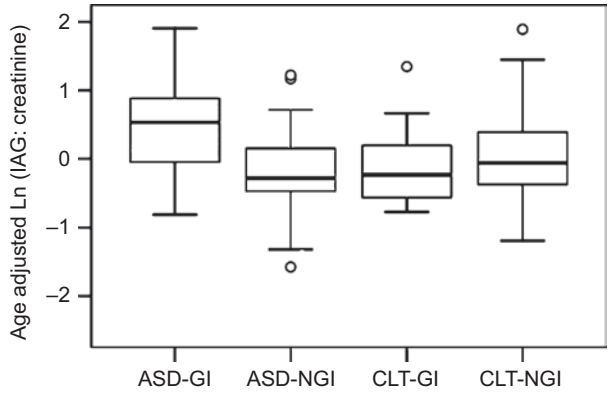

Figure 4. Box plot of the distribution of indolyl-3-acryloylglycine (IAG) (age-adjusted natural logarithm of IAG:creatinine ratio) in the autism spectrum disorder (ASD) and combined control groups (siblings, SIB and healthy controls, CON) with and without ongoing gastrointestinal (GI) disturbance. ASD-GI, children with ASD with GI disturbance; ASD-NGI, children with ASD without GI disturbance; CLT-GI, combined control groups (SIB+CON) with GI disturbance; CLT-NGI, combined control groups (SIB+CON) without GI disturbance.

The caregiver questionnaire revealed that 46% of the ASD participants had 'GI disturbance', i.e. ASD-GI. Caregivers reported that a further 46% did not experience ongoing GI issues, i.e. ASD-NGI. For the remaining 8%, caregivers reported they were 'unsure' whether their child experienced ongoing GI issues. Twenty-six per cent of caregivers of control participants of the SIB group and 5% of the CON group reported chronic GI disturbance (Figure 3). In subsequent analyses, individuals unsure about GI status were excluded.

An analysis of covariance including age as a covariate found that there were statistically significant higher urinary IAG levels and IAG:creatinine ratio in the ASD participants with ongoing GI problems compared with ASD participants without GI problems ($p=0.006$ and $p=0.001$, respectively) (Figure 4). However, there was no statistically significant association between either urinary IAG or IAG:creatinine ratio and the presence of GI disturbance in the combined control cohort ($p=0.283$ and $p=0.364$, respectively). Both the IAG levels and IAG:creatinine ratios were statistically significantly higher in ASD individuals with GI symptoms than the control (combined SIB and CON groups) individuals with GI symptoms.

Discussion

The key finding from this study was that there were significantly higher urinary IAG levels and IAG:creatinine ratios in children with ASD whose caregivers reported the presence of GI disturbance compared with ASD children whose caregivers reported the absence of ongoing GI issues. It is not clear whether this is a consequence of the GI pathology or a factor that is contributing to the GI symptoms. There is also an issue of whether raised IAG levels are specific to ASD with ongoing GI problems. IAG did not differ between controls (SIB and CON groups) with and without

ongoing GI problems. Additionally, ASD individuals with ongoing GI problems had statistically significantly higher IAG levels than control individuals with ongoing GI problems. This suggests that the raised IAG levels may be specific to autism, but further research is warranted to reproduce this finding and specifically assess whether the IAG plays a causative role in GI disturbance in ASD.

Given the heterogeneity of the aetiology and pathophysiology of ASD it is therefore plausible that the anomalies that are evident with respect to IAG and the IAG:creatinine ratio may provide insights into the aetiology and pathophysiology of specific ASD subtypes. Therefore, while IAG is not useful as a diagnostic biomarker *per se*, it may be a useful biomarker for ASD with associated GI disturbance. This may assist with the selection of targeted interventions that normalise GI disturbance such as probiotics, prebiotics and/or antibiotics. Such interventions may in turn have the potential to mitigate the manifestations of autism. It is also possible that IAG may be a useful biomarker to monitor response to such interventions.

As with previous studies (Horvath & Perman 2002a, b), the proportion of children with ongoing GI issues was greater in the ASD group compared with control groups. Additionally, the frequency of GI issues in the sibling controls was intermediate between the ASD and the community controls, which is concordant with a previous study (Horvath & Perman 2002b). It is clear that autism has a genetic basis from twin studies whereby 60–90% of cases have concordant expression of autism or a related disorder (Veenstra-VanderWeele & Cook 2004). The rate of sibling recurrence of ASD is up to 8% (Muhle et al. 2004). It is therefore conceivable that the intermediate frequency of GI disturbance which was observed in the SIB group compared with the ASD and the CON groups is genetically predetermined.

There was no statistically significant difference between the mean IAG level and IAG:creatinine ratio in the three groups, although there was a trend towards the IAG:creatinine ratio being higher in the ASD group compared with the two control groups. This is concordant with the study by Wright et al. (2005). The mean urinary IAG concentration in our study was very similar to the results of Wright et al. (2005). When our IAG:creatinine results were transformed using the same approach as Wright et al. (2005) without age adjustment, the urinary IAG:creatinine ratios (mean ± SD) for the ASD and combined controls were -5.38 ± 0.61 and -5.62 ± 0.50, respectively, compared with the report of -5.29 ± 0.62 and -5.25 ± 0.48 for an ASD and control cohort, respectively (Wright et al. 2005). However, as Bull et al. (2003) reported IAG levels as the area under the peak rather than an absolute concentration, it is not possible to directly compare the levels between studies.

A previous study examining age variations of IAG excretion versus urinary creatinine levels in healthy children showed that the ratio of urinary IAG (mg):creatinine was much higher in children aged 2–6 years but decreased to lower levels and remained low until advanced age (Marklova & Hais 1978). In our study, urinary IAG:creatinine had a statistically significant negative correlation with the age of the participants due to the strong association between age and urinary creatinine concentration. Furthermore, a trend towards a decline in urinary IAG:creatinine with age in ASD children was also found by Wright et al. (2005), but it was not statistically significant. Bull et al. (2003) found no relationship with age, but their cohort did not include very young children. Due to the potential confounding nature of age, age was included as a covariate in the analysis of covariance tests, as was the case with the study by Wright et al. (2005).

It is generally accepted that the excretion of creatinine is relatively fixed over time and for that reason urinary metabolites are reported as a ratio of creatinine. We elected to normalise urinary IAG to creatinine thus enabling differences in urinary dilution to be corrected. However, it was notable that the urinary creatinine concentrations in the CON group were significantly higher than all other groups when compared separately. Our findings are congruent with a previous study where urinary creatinine was reported to be lower in children with PDDs compared with controls (Whiteley et al. 2006). Impaired growth or significant differences in muscle mass were not prevalent in children with ASD compared with those without ASD. Thus, neither condition was a likely explanation for the reduced urinary creatinine excretion seen in the ASD group (Adams-Chapman & Stoll 2006). In our study, we also found creatinine had a strong association with age which is expected as muscle mass increases with age in childhood. Due to the potential confounding nature of age, age was included as a covariate in the analysis of covariance tests, as was the case with the study by Wright et al. (2005). Thus although it seems that use of creatinine to normalise urinary IAG concentrations is problematic, it is the standard method used and the results from this study are consistent whether urinary IAG concentration or urinary IAG:creatinine ratio is used.

Of the 26 children with ASD who had GI disturbance, 10 were implementing a gluten-free diet. There was no clear correlation between urinary IAG levels and whether the ASD-GI participants implemented a gluten-free diet or not. This finding is consistent with an earlier study by Whiteley et al. (1999) who found no significant changes in urinary IAG levels in participants before and after a period of gluten-free dietary intervention. Hence it can be concluded that participants' IAG levels in the current

study were unlikely to be affected by implementation of a gluten-free diet.

There was no statistically significant difference in the urinary IAG or IAG:creatinine ratio between males and females in this study. Differences in urinary IAG excretion between genders have not previously been reported in the literature. Thus, although the gender distribution of the ASD sample was 5:1 (male:female) compared with 1:1 among controls, our study sample's gender ratio, was consistent with literature reports of the ASD gender ratio. It is therefore unlikely that this has significantly biased the comparison.

It should be noted that in our study, both typically developing siblings and unrelated community children were selected as distinct control groups. Previously, other studies stated their controls were children without autism but it is not clear whether the controls were unrelated to the ASD participants or ASD participants' siblings, or a mixed cohort (Bull et al. 2003, Wright et al. 2005). Given the genetic basis of autism, it is important that sibling controls and unrelated controls are considered as distinct comparator groups.

A limitation of this study is the observational cross-sectional study design that does not allow determination of whether IAG levels pre-exist and influence GI disturbance in ASD or are only a marker of the disturbance. Similarly, it would be useful for future studies to utilise more objective measures of illness severity in functional gastrointestinal disorders. Study of IAG in other groups with GI disturbance will reveal greater insight into whether raised IAG levels are specific to GI disturbance in autism. Due to the small number of individuals with AS it was not possible to determine whether the relationship of IAG with GI disturbance differed between individuals with AD and AS.

In conclusion, this study reports for the first time significantly higher urinary IAG levels and IAG:creatinine ratio in children with ASD whose caregivers reported the presence of GI disturbance compared with children with ASD without ongoing GI issues. Additionally, the IAG levels were higher in children with ASD and ongoing GI issues than in control children with ongoing GI issues, suggesting that the raised levels may be specific to autism. Further research is required to replicate these findings, directly study the specificity of IAG to GI disturbance in autism, and to determine whether IAG plays a causal role in GI disturbance of autism.

Urinary IAG and the IAG:creatinine ratios do not appear useful as biomarkers for distinguishing between children with and without ASD. Therefore, IAG may have more potential use in ASD by providing pathophysiological insight into the GI symptoms that are common with children with ASD and thereby aiding in the development and selection of interventions and monitoring of response.

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