Indolyl-3-acryloylglycine (IAG) is a putative diagnostic urinary marker for autism spectrum disorders

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Summary

Background: Autism is a heterogeneous pervasive developmental disorder with a poorly defined aetiology and pathophysiology. There are indications that the incidence of the disease is rising but still no definitive diagnostic biochemical markers have been isolated. Here we have addressed the hypothesis that urinary levels of trans-indolyl-3-acryloylglycine (IAG) are abnormal in patients diagnosed with autism spectrum disorders (ASD) compared to age-matched controls.

Material/Methods: Urine samples were collected on an opportunistic basis and analysed for IAG concentration (normalised against creatinine content to account for changes in urinary volume) using reversed phase HPLC with UV detection.

Results: Statistical analysis (Mann-Whitney tests) showed highly significant increases (p=0.0002) in the levels of urinary IAG in the ASD group (median 942 µV per mmol/L of creatinine [interquartile range 521–1729], n=22) compared to asymptomatic controls (331 [163–456], n=18). Detailed retrospective analysis showed that gender (boys 625 µV per mmol/L of creatinine [294–1133], n=29; girls 460 [282–1193], n=11: P=0.79) and age (control donor median 10 years [8–14], n=15; ASD median 9 years [7–11] n=22: P=0.54) were not significantly correlated with IAG levels in this non-blinded volunteer study.

Conclusions: Our results strongly suggest that urinary titres of IAG may constitute an objective diagnostic indicator for ASD. Mechanisms for the involvement of IAG in ASD are discussed together with future strategies to address its specificity.

key words: Autism • trans-indolyl-3-acryloylglycine (IAG) • diagnosis of autistic spectrum disorders • urinary creatinine • reversed phase HPLC with UV detection • developmental disease markers


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BACKGROUND

Autism, a heterogeneous condition, is categorised as a pervasive developmental disorder usually present within the first three years of infancy. Symptoms include qualitative impairments in language and communication, problems in reciprocal social interaction and the presence of repetitive or stereotyped behaviours [1]. Studies indicating the possibility of a worldwide increase in the numbers of diagnosed cases of autism spectrum disorders [2] (ASD) [3–5] have further fuelled the need for more research into the causation and proliferation of autism spectrum disorders (ASD). Although much of this increase has been ascribed to improved diagnostic criteria and better awareness of the syndrome, there still remain no reproducible genetic or biological markers, or objective medical tests for the diagnosis of autism.

We have refined methods that have revealed the presence of trans-indolyl-3-acyryloylglycine (IAG) in the urine of patients with autism spectrum disorders [6]. Although IAG is unequivocally present in the urine of asymptomatic subjects [7], it is elevated in some disease states such as Hartnup disease [8] and light sensitive dermatitis [9,10]. Others have reported marked fluctuations in urinary titres with age: Marklova et al. [7] observed that maximum urinary IAG excretion was found in subjects aged between 2-7 years. Here we address the important hypothesis that the presence of repetitive or stereotyped behaviours [1]. Studies indicating the possibility of a worldwide increase in the numbers of diagnosed cases of autism spectrum disorders (ASD). Although much of this increase has been ascribed to improved diagnostic criteria and better awareness of the syndrome, there still remain no reproducible genetic or biological markers, or objective medical tests for the diagnosis of autism.

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MATERIAL AND METHODS

Subjects

The research was carried out in accordance with the Declaration of Helsinki and with the authorisation of the University of Sunderland Ethics Committee.

Samples were collected unblinded from two groups:
a) Autism Spectrum Disorder (ASD) diagnosed using DSM-IV/ICD-10 criteria (n=22);
b) Control (no diagnosis) (n=18).

Subjects were recruited as follows:
Patients were required to have received a formal diagnosis of ASD made by a qualified clinician using standardised DSM-IV or ICD-10 diagnostic criteria. Subjects (median age 9 years, range 6–22) were not on any medication or special dietary intervention at the time of urine testing. Samples were collected from patients (before noon, between July 1998 – September 1998). Patient's reports did not indicate any connection between symptom onset and the administration of the combined Measles-Mumps-Rubella (MMR) vaccination.

Control (median donor age 10 years, range 5–18) samples taken from asymptomatic guests at a birthday parties (early afternoon between August 1998 – October 1998) not diagnosed with any Pervasive Developmental Disorder or involved on any specific dietary/drug intervention.

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Statistical analysis

Data are reported as median (interquartile range or IQR) unless otherwise stated. All statistical analyses and tests were conducted using GraphPad 3.0 software (GraphPad California, USA). The alpha value for statistical significance in (two-tailed) hypothesis or correlation testing was P<0.05.

RESULTS

IAG titres, normalised for creatinine content (to eliminate fluctuations in urine volume) were clearly not normally distributed (Figure 1A). Control median levels of IAG were 331.3 (IQR 163–456) µV per mmol/L compared to 941.9 (521–1729) µV per mmol/L. A Mann Whitney 2-tailed ranks test showed that there was a highly significant difference in quantitative levels of urinary IAG between ASD and control groups (P=0.0002, see Figure 1A).

Due to the skewing of sex ratios in autism (predominantly boys 4:1) it was important to establish if there was any connection between gender and levels of IAG excreted. To test the hypothesis that males excrete significantly different amounts of IAG to females, subjects
were divided into subgroups containing males and females irrespective of disease state and the data were analysed using a Mann Whitney test. The results (Figure 1D) showed that there was no significant difference between gender and IAG levels irrespective of disease state: male median 626 (294–1133), n=29 compared to female median 460 (282–1193), n=11: P=0.79.

There were no significant differences in the age of the donors in each group: control median 10 (interquartile range 8–14) versus ASD 9 years (7–11); P=0.54, Figure 1B.

Spearman’s correlation statistic (two-tailed test) was used to test for any relationship between small age differences at time of sampling and urinary IAG. Results (regression coefficient=-0.069; P=0.68; Figure 1C) showed that at the 95% significance level, no relationship was found between age and IAG titres for all the donors (across patient and control groups) prepared to reveal their age.
Despite the small sample sizes, it seems that neither age nor gender appears to contribute to the overall observed differences between ASD and control groups.

**DISCUSSION**

Our own unpublished preliminary observations have indicated that urinary IAG levels were elevated in subjects diagnosed with ASD compared to control groups. This quantitative study has shown highly significant differences in the levels of urinary IAG as a function of creatinine content between these groups. Urine samples are relatively easy to obtain in paediatric illness and hence represent an ideal diagnostic test. Despite this, the sampling regime used here contained minor flaws. Control urine samples were collected later in the day than those donated by patients. The use of creatinine to normalise for urine concentration helps to overcome this and it has been shown that dietary factors are relatively unimportant in dictating IAG levels in small human cohorts [10]. IAG levels have been reported to fluctuate slightly in a seasonal manner [11] but our samples were collected within a closely overlapping time period (none of the samples were taken within the winter period when IAG concentrations were elevated). The magnitude of the shifts here are much greater than those which might be attributable to dietary, metabolic or sampling vagaries in earlier studies. However, the use of urine has intrinsic flaws as an indicator of pathophysiological markers. We intend to seek evidence that the titres of IAG are abnormal in the systemic circulation too. The availability of patient serum for studies (the autism genetic resource exchange, founded by ‘Cure Autism Now’ will release genetic information and serum samples from affected family groups) of this nature will allow us to measure titres of both IAG and its non-conjugated precursor indole-3-acrylic acid in the systemic circulation. In order to confirm the relevance of IAG as a possible diagnostic marker for autism spectrum disorders, we intend to conduct double blind trials using much larger patient cohorts. Further research is needed as to the exact source and role of IAG in autism and the relationship, if any, between IAG and the severity of symptoms observed in the autistic syndrome.

The aetiology of ASD remains an enigma. In the absence of marked morphological defects in post-mortem brain tissues from autistic patients, signalling defects in neurotransmitter or neuromodulatory pathways are now attracting considerable attention. For example, GABA levels are elevated in ASD serum [12]; several opiate-like peptides (besides IAG) are elevated in ASD urine [e.g. 13] and serotonergic pathways have been implicated too [14]. Serotonin uptake inhibitors (SSRIs) are used by paediatricians and clinical psychologists to treat the symptoms of ASD although more rigorous/large controlled clinical trials are needed [reviewed in 15]. The exact route of formation for IAG is, as yet, unclear but it may be linked to the conversion of tryptophan to 5-hydroxy tryptamine (5-HTP) [16].

If IAG is to be used as a diagnostic factor for autism it is crucial that future studies address the specificity of the molecule for this disease. Linkage to Hartnup’s disease and polymorphous light dermatosis has been established [8,9] (although these themes have not been revisited for some years) but no systematic studies have been conducted on other paediatric diseases of the CNS. In this respect, we have heard anecdotal reports (based on very small patient cohorts or individuals submitting samples prior to definitive diagnosis) that urinary IAG is elevated in Attention-Deficit Hyperactivity Disorder (ADHD) and in dyslexia. We plan to garner some hard statistics on these issues but we will need support from both paediatricians and parents to make this possible.

**CONCLUSIONS**

Here we report a sensitive bioassay method applicable to readily accessible urine samples that can detect (highly significant) abnormally high levels of IAG in ASD. Further studies are urgently required to assess the specificity & relevance of this substance as a diagnostic marker and whether it is a determinant of disease severity in autism.

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