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ORIGINAL PAPER

Distinct Plasma Profile of Polar Neutral Amino Acids, Leucine, and Glutamate in Children with Autism Spectrum Disorders

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Abstract The goal of this investigation was to examine plasma amino acid (AA) levels in children with Autism Spectrum Disorders (ASD, N = 27) and neuro-typically developing controls (N = 20). We observed reduced plasma levels of most polar neutral AA and leucine in children with ASD. This AA profile conferred significant post hoc power for discriminating children with ASD from healthy children. Furthermore, statistical correlations suggested the lack of a typical decrease of glutamate and aspartate with age, and a non-typical increase of isoleucine and lysine with age in the ASD group. Findings from this limited prospective study warrant further examination of plasma AA levels in larger cross-sectional and longitudinal cohorts to adequately assess for relationships with developmental and clinical features of ASD.

Keywords Blood · Predictive value · Polar neutral amino acids · Leucine · Glutamate

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Introduction

Autism Spectrum Disorders (ASD), which include autism, Asperger's disorder, and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), are neuro-psychiatric developmental conditions of varying severity characterized by social deficits, impairments in verbal/nonverbal communication, and repetitive/restrictive patterns of behavior. Children with autism exhibit an abnormal pattern of early brain growth (Hardan et al. 2001; Courchesne et al. 2003; Hazlett et al. 2005; Courchesne et al. 2007). Comorbid behavioral and neurological symptoms, including epilepsy, sleep disturbances, severe irritability/aggression and mood instability, are common (Geschwind 2009). A number of systemic abnormalities have been suggested, including increased vulnerability to oxidative stress and immune dysfunction (Ratajczak 2011).

Amino acid (AA) abnormalities have received less attention in ASD despite several investigations implicating them in other neuro-psychiatic disorders. Elevated plasma levels of serine have been observed in patients with schizophrenia (Sumiyoshi et al. 2004). In addition, elevated plasma

levels of serine as well as of the inhibitory AA taurine and decreased levels of the excitatory AA glutamate were observed in a study of individuals with depression (Altamura et al. 1995). Studies have also examined patients with mood and comorbid anxiety disorders and observed that individuals with obsessive compulsive disorder and a coexisting major depressive disorder (OCD-MDD) had lower plasma tryptophan compared to OCD patients without MDD (Bellodi et al. 1997). These observations point to a potential contribution of AA to the pathophysiology of neuro-psychiatric disorders and warrant their investigation in ASD.

Here we report results from a cross-sectional pilot study of the plasma AA profile in patients with ASD. The goals of this study were: (1) to determine whether children with ASD had altered levels of plasma AA compared to neuro-typically developing healthy children (HC); (2) to explore potential differences in the developmental pattern of plasma AA between children with ASD and HC children; and (3) to determine whether children with ASD could be distinguished from HC children based on their plasma AA profile.

Methods

Participants

Blood samples were obtained from 47 children (age range: 3–12 years): 27 with ASD and 20 healthy controls (HC). Subjects with ASD were referred to our research clinic from the community and met the following inclusion criteria: (1) diagnosis of ASD through expert clinical evaluation and two structured research diagnostic instruments, namely the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter et al. 1994) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 1989); and (2) absence of any neurological and genetic disorders. Those with autism met both ADI-R and ADOS criteria for autism. Subjects with PDD-NOS had ADOS scores ranging from 7 to 10 while meeting ADI-R criteria for autism. Children with secondary autism such as those with tuberous sclerosis or Fragile X syndrome were excluded from the study.

The control group consisted of medically healthy children (HC) recruited from communities through advertisements in areas socially and economically comparable to the communities from which ASD subjects originated. Control subjects were screened by face-to-face interviews, questionnaires, telephone interviews, and observation during psychometric tests. All control subjects had no present or lifetime history of psychiatric disorders as assessed by the Schedule for Affective Disorders and Schizophrenia for School-Age Children (Kaufman et al. 1997). Cognitive testing was available on some but not all children. After procedures were fully explained, parents of all participants or their legal guardians provided written informed consent. Verbal assent was obtained from participants age 6 and older when children are able to understand study procedures. The University Institutional Review Board approved the methodology for the study. Demographic characteristics of ASD and HC groups are summarized in Table 1.

Plasma Sample Preparation

Blood was drawn by venipuncture into K_2 EDTA BD Vacutainer® tubes (BD Biosciences, San Jose, CA) and placed immediately on ice. Blood was centrifuged at $400\times g$ for 10 min at 4°C to pellet leukocytes and erythrocytes. The resulting supernatant (platelet-rich plasma) was transferred into a new tube and centrifuged at $3{,}000\times g$ for 10 min at 4°C to pellet platelets. This procedure typically results in <1–2% residual platelets in the supernatant (herein referred to as platelet-poor plasma). Platelet-poor plasma was then transferred into cryogenic vials and frozen at −80°C until the day of analysis.

Table 1 Subject demographics

Characteristics	ASD group	HC group	*p*
N	27	20	–
Age in months (mean ± SD)	84 ± 28	88 ± 30	NS
Age range (months)	37–128	41–151	NS
Ethnicity (A/B/C/H[a])	4/5/15/3	1/3/14/2	NS
Gender ratio (F/M[b])	6/21	11/9	.02
FSIQ[c] (mean ± SD)	70.1 ± 24.0	114 ± 11.6	$<10^{-4}$

Shown are summary statistics for Autism Spectrum Disorders (ASD) and healthy control (HC) groups and *p* values for evaluation of characteristics differences between the cohorts

[a] stands for Asian/Biracial/Caucausian/Hispanic

[b] stands for Female/Male

[c] stands for full scale IQ and was available on 18 out of 27 ASD and 19 out of 20 HC subjects

Amino Acid Quantitation

Frozen platelet-poor plasma samples were thawed on ice. One hundred microliters of sample were used for analysis. Quantitation of AA was performed using the EZ:faast™ kit (Phenomenex, Torrance, CA), a recently-developed sensitive and reliable method for AA analysis (Badawy et al. 2008; Mohabbat and Drew 2008). The EZ:faast™ procedure was performed according to manufacturer's instructions with modifications for LC–MS analysis. Samples were spiked with a mixture of internal standards (homoarginine, methionine-d3, and homophenylalanine), extracted, and derivatized with propyl chloroformate. Organic phase was evaporated using a vacuum concentrator and the residue was redissolved in 50% methanol. Samples were analyzed by LC–MS using Agilent 1100 chromatograph (Agilent, Santa Clara, CA) coupled to Quattro Premier triple quadrupole mass spectrometer (Waters, Milford, MA). One microliter of sample was injected on 2.1 × 250 mm 4 μm amino acid analysis column (Phenomenex) at 0.25 mL/min flow rate. The column was held at room temperature. Mobile phase A was 10 mM ammonium formate in water; mobile phase B was 10 mM ammonium formate in methanol. Derivatized AA were separated using a 13 min gradient elution from 70 to 85% of mobile phase B. Detection was performed in positive electrospray ionization mode using selected reaction monitoring of transitions, as specified by the manufacturer of the EZ:faast™ kit. The transitions were confirmed and optimized for mass spectrometer using derivatized AA standards. Quantitation of AA was performed using internal calibration with internal standard correction using QuantLynx software (Waters).

Statistical Analysis

All statistical analyses were performed using the JMP8 software (SAS Institute, Cary, NC). The normality of AA data was tested using the Shapiro–Wilk test and due to the lack of normality of some AA, non-parametric statistics were chosen to describe AA levels in each group. Hence, individual AA measures are presented as median (50th percentile) and interquartile range [25th percentile, 75th percentile]. Between-group comparisons (ASD vs. HC) of AA levels were performed using the non-parametric Wilcoxon rank sum test. Pairwise correlations between continuous measures (AA levels, age) within each group were evaluated using the non-parametric Spearman ρ test. Differences and correlations were considered significant with $p < .05$. However, for multiple comparisons, we used the Bonferroni correction (.05 divided by the number of simultaneous tests). To assess the predictive values of AA measures for group identification (ASD vs. HC), the choice of single predictors and combinations of predictors was made by discriminant analysis (F test, with stepwise variable selection). Predictive abilities were expressed as the area under the receiver operating characteristics (ROC) curve, which plots the frequency of true positive (sensitivity) against the frequency of false-positive (1-specificity) results. Corresponding p values were calculated by the χ^2 test. To assess potential effects of gender and ethnicity, combined with patient group, on AA levels, we performed covariate analyses by stepwise regression for each AA measured with corresponding p values calculated by the F test.

Results

Children with ASD Show Significant Differences in Plasma Levels of Several AA

We quantified AA in platelet-poor plasma obtained by a dual centrifugation procedure that yields fluid with minimal contamination from erythrocyte, leukocyte, and platelet intracellular compartments, hence providing a reliable assessment of AA levels. Table 2 lists medians and interquartile ranges for 20 AA quantified by LC–MS in ASD and HC groups, as well as p values for between-group comparisons. Distributions of AA plasma levels are shown in Fig. 1. After controlling for multiple comparisons, 7 out of the 20 AA measured were significantly decreased in children with ASD ($p < .0025$), namely glutamine (GLN), threonine (THR), asparagine (ASN), citrulline (CIT), serine (SER), tyrosine (TYR), and leucine (LEU). Six more AA showed a trend towards significant differences between the two groups, with a p value between .0025 and .05. Among them, glutamate (GLU) was the only AA for which levels were higher in the ASD group compared to the control group.

Neither gender nor ethnicity impacted AA levels, when examined either alone in univariate analyses or along with subject group in covariate analyses. Within the ASD group, patients with PDD-NOS were not different from patients with autism for any AA measured. Children with ASD taking prescription medications were not different from those without medications for any AA measured. Within the ASD group, no significant correlations were found between ADI-R and ADOS scores and any AA measured. Within either ASD or HC groups, no significant correlations were found between full scale IQ scores and any AA measured. Finally, the time of the blood draw (ranging from 10 am to 2 pm for both groups) did not significantly affect the levels of plasma AA within each group.

Polar Neutral AA are the Most Affected AA Group in Children With ASD

Based on biophysical and biochemical AA classification, polar neutral AA represented the most affected AA group

(6 out of 7 measured) in children with ASD. Plasma levels of polar neutral AA, except TYR, correlated highly with each other in both ASD and HC groups, emphasizing the relevance of this group classification (Table 3). Among polar neutral AA, GLN, SER, and ASN were the most highly correlated with each other in the ASD group and to a lesser extent in the HC group. Other AA groups (e.g., branched chain, hydrophobic, and essential) were not overall affected in the ASD group to the extent that the polar neutral AA group was.

Glutamate and Other AA Show Differential Relationship to Age in ASD and HC Groups

Since ASD is a developmental disorder, we explored potential relationships between plasma AA levels and age in ASD and HC groups. Based on our cross-sectional analysis of ASD and HC children, we found that plasma levels of glutamate (GLU) and aspartate (ASP) were inversely correlated with age in the HC group, but not in the ASD group (Fig. 2). Conversely, plasma levels of isoleucine (ILE) and lysine (LYS) were directly correlated with age in the ASD group, but not in the HC group (Fig. 2).

Fig. 1 Distribution of plasma amino acid (AA) levels in subjects with Autism Spectrum Disorders (ASD, N = 27) and healthy control (HC, N = 20) subjects. Shown are *box plots* delimited by 25th and 75th percentile (interquartile range), with median *line* within the box. ASD subjects comprise patients with autism (N = 22) and patients with Asperger's disorder or PDD-NOS (N = 5). There were no differences in plasma AA levels between patients with autism and patients with Asperger's disorder or PDD-NOS. *Open symbols* represent females and *filled symbols* represent males. There were no differences in AA levels between genders in both ASD and HC groups

Plasma AA Levels Provide Significant Predictive Value for Discriminating Children With ASD From HC subjects

Predictive values of AA measures for distinguishing ASD patients from HC subjects were also examined. *Post hoc* discriminant analysis, using stepwise variable selection for F test, was performed for the seven AA measures that were significantly different between the ASD and HC groups.

Table 2 Amino acid (AA) levels in subjects with Autism Spectrum Disorders (ASD) and healthy control (HC) subjects

AA	Plasma level in μM (median [interquartile range])		*p*
	ASD	HC	
GLN	**84.6 [70.0; 95.5]**	**112 [95.5; 131]**	**$<10^{-4}$**
THR	**62.0 [51.2; 78.8]**	**87.1 [67.0; 111]**	**.0006**
ASN	**5.95 [4.88; 7.63]**	**8.66 [7.03; 11.3]**	**.0006**
CIT	**17.9 [13.8; 26.7]**	**27.8 [22.5; 31.1]**	**.0006**
SER	**82.5 [65.3; 103]**	**118 [91.1; 131]**	**.001**
TYR	**109 [85.4; 118]**	**151 [107; 168]**	**.001**
LEU	**259 [224; 306]**	**338 [293; 459]**	**.002**
PHE	126 [105; 141]	142 [126; 214]	.004
TRP	14.6 [11.3; 16.9]	19.6 [15.4; 23.5]	.006
GLU	97.2 [73.6; 130]	74.8 [56.7; 105]	.02
HIS	177 [158; 208]	197 [177; 238]	.02
MET	42.6 [35.8; 51.4]	53.2 [40.3; 67.6]	.03
ILE	169 [139; 196]	194 [154; 272]	.05
LYS	315 [265; 376]	376 [294; 450]	.1
VAL	391 [321; 427]	445 [332; 544]	.2
ARG	190 [126; 216]	191 [165; 282]	.3
PRO	437 [331; 602]	528 [403; 665]	.3
GLY	181 [151; 230]	196 [157; 216]	.8
ALA	709 [618; 834]	725 [579; 821]	.9
ASP	3.03 [2.58; 3.48]	3.41 [2.13; 4.07]	1

AA levels in plasma (μM) are indicated as median values (with interquartile range—i.e., 25th and 75th percentiles in brackets) and ranked by increasing *p* value for the between-group difference (Wilcoxon rank sum test). Non-parametric statistics were used due to the unequal number of subjects in the 2 groups and lack of normal distribution for some AA. For AA indicated in bold, *p* values are below the Bonferroni correction cutoff for multiple analyses ($p = .0025$ for 20 AA)

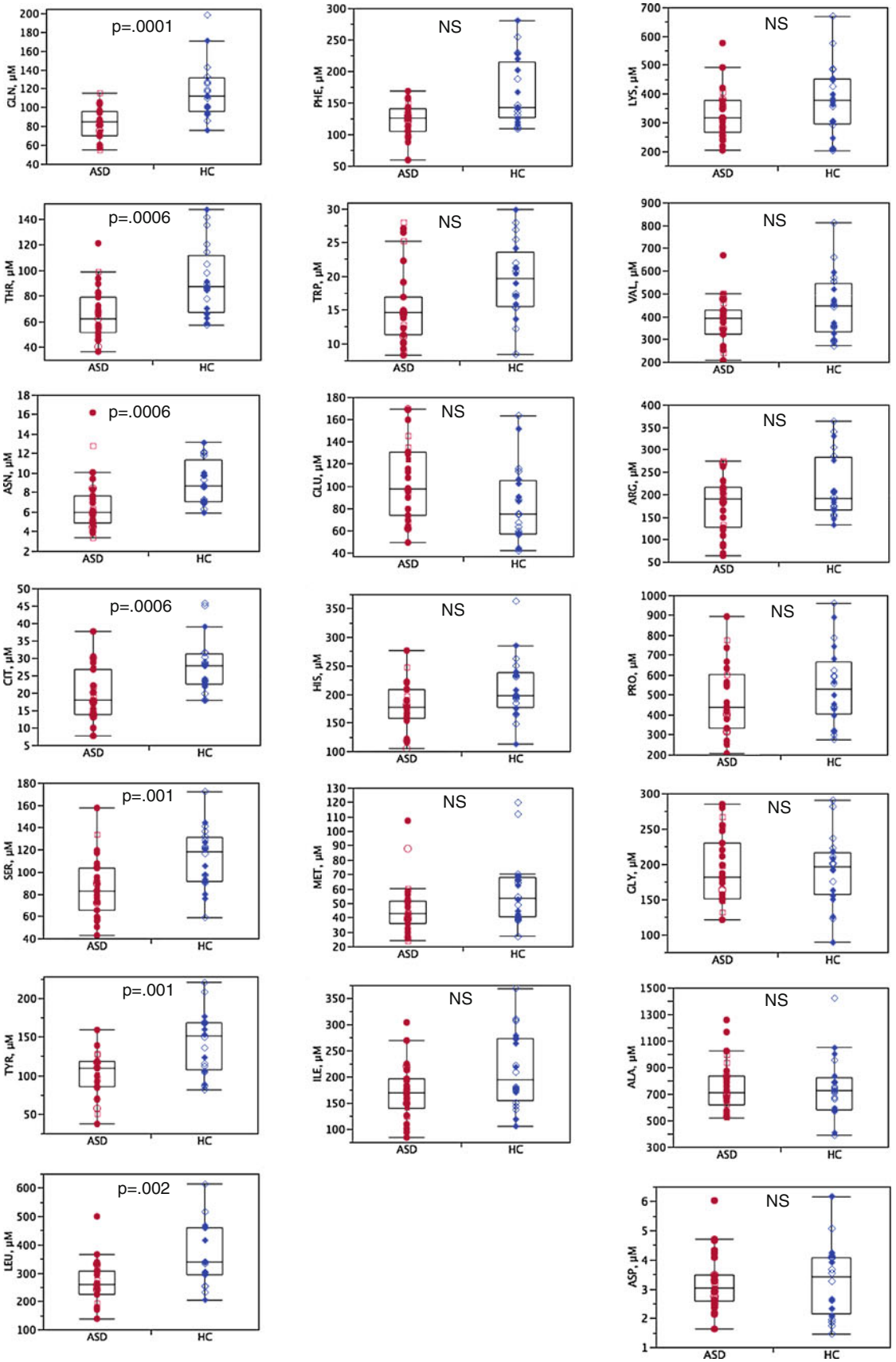
p=.0001
GLN, µM
ASD
HC
NS
PHE, µM
NS
LYS, µM
p=.0006
THR, µM
NS
TRP, µM
NS
VAL, µM
p=.0006
ASN, µM
NS
GLU, µM
NS
ARG, µM
p=.0006
CIT, µM
NS
HIS, µM
NS
PRO, µM
p=.001
SER, µM
NS
MET, µM
NS
GLY, µM
p=.001
TYR, µM
NS
ILE, µM
NS
ALA, µM
p=.002
LEU, µM
NS
ASP, µM

Table 3 Correlations among levels of polar neutral amino acids (AA) in Autism Spectrum Disorders (ASD) and healthy control (HC) groups

AA	THR	ASN	CIT	SER	TYR
ASD group					
GLN	.56 (.003)	.75 ($<10^{-4}$)	.59 (.001)	.74 ($<10^{-4}$)	NS
THR		.65 (.0003)	.63 (.0004)	.56 (.003)	NS
ASN			.55 (.003)	.69 ($<10^{-4}$)	NS
CIT				.59 (.001)	NS
SER					NS
HC group					
GLN	.74 (.0002)	.74 (.0002)	.49 (.03)	.60 (.005)	NS
THR		.71 (.0004)	NS	.80 ($<10^{-4}$)	NS
ASN			NS	.46 (.04)	NS
CIT				.60 (.005)	NS
SER					NS

Spearman's ρ coefficients (with a corresponding p value) are indicated for each AA pair. *NS* indicates lack of significant correlation

The predictive values for ASD versus HC subject discrimination were expressed as areas under the ROC curve, as indicated in Table 4. Among single AA measures, the best value was obtained with GLN, with an area under the ROC curve of .87 (1 is the maximum) with $p < 10^{-4}$. Combinations of GLN × TYR, and GLN × TYR × ASN resulted in areas under the ROC curve of .92 and .96, respectively, with $p < 10^{-4}$ for both. Combinations of GLN with the non-polar neutral AA LEU and GLU also provided excellent predictive values for discriminating between ASD and HC groups (area under the ROC curve of .92, $p < 10^{-4}$ for both).

Discussion

In this study, we compared the levels of 20 AA in platelet-poor plasma of children with ASD and healthy controls, using a highly sensitive, standardized method for AA quantitation by mass spectrometry. We found significantly lower plasma levels for seven AA (GLN, THR, ASN, CIT, SER, TYR, and LEU, ranked in decreasing order of significance) in the ASD group compared to the HC group. Additionally, we observed a different pattern of plasma levels of four other AA (GLU, ASP, ILE, and LYS) with respect to age in the ASD group compared to the HC group. Finally, we found that plasma levels of several polar neutral AA, either individually or combined, yielded very good to excellent predictive value for discriminating ASD from HC subjects.

Plasma AA levels have been previously measured in patients with ASD (Moreno-Fuenmayor et al. 1996; Aldred et al. 2003; Shinohe et al. 2006). However, no particular pattern of AA abnormalities characteristic of ASD had emerged. Differences in analytical and statistical methodologies, age of subjects, selection of control cohorts versus external reference values, and biological sample preparations may have contributed to the discrepancies in reported AA levels in ASD, as summarized in Table 5.

In our study, we measured AA levels in platelet-poor plasma prepared by dual centrifugation of whole blood. Platelet-poor plasma differs in composition from platelet-rich plasma or serum (Banks et al. 2005). Among these three types of preparations, platelet-poor plasma provides the most accurate appraisal of AA levels, since serum is contaminated with products of coagulation and concomitant platelet, leukocyte and erythrocyte activation, whereas platelet-rich plasma that has been frozen and thawed is contaminated with intra-platelet contents due to platelet lysis. Since platelets contain several AA, including glutamate and aspartate (Rolf et al. 1993; Tremolizzo et al. 2006), the use of platelet-poor plasma, but not of other preparations, minimizes non-specific AA release during sample preparation.

Extracellular GLU is regarded as a potentially important factor in the etiology of ASD due to its excitotoxic capacity in the brain (Blaylock and Strunecka 2009). A previous study suggested that serum levels of GLU were significantly increased in adults with autism compared to healthy controls and weakly correlated with disease severity based on the ADI-R social score (Shinohe et al. 2006). Here, we found only slightly increased GLU levels in the ASD group compared to the HC group based on platelet-poor plasma measurements, in children. This difference did not reach statistical significance after correction for multiple comparisons. Interestingly, plasma GLU levels are developmentally regulated (Lepage et al. 1997). We observed age-dependent decrease in plasma GLU (as well as ASP) in the HC, but not in the ASD cohort. This pattern is suggestive of the lack of normal developmental downregulation of extracellular GLU levels in plasma of ASD patients, which may reach statistically significant difference from

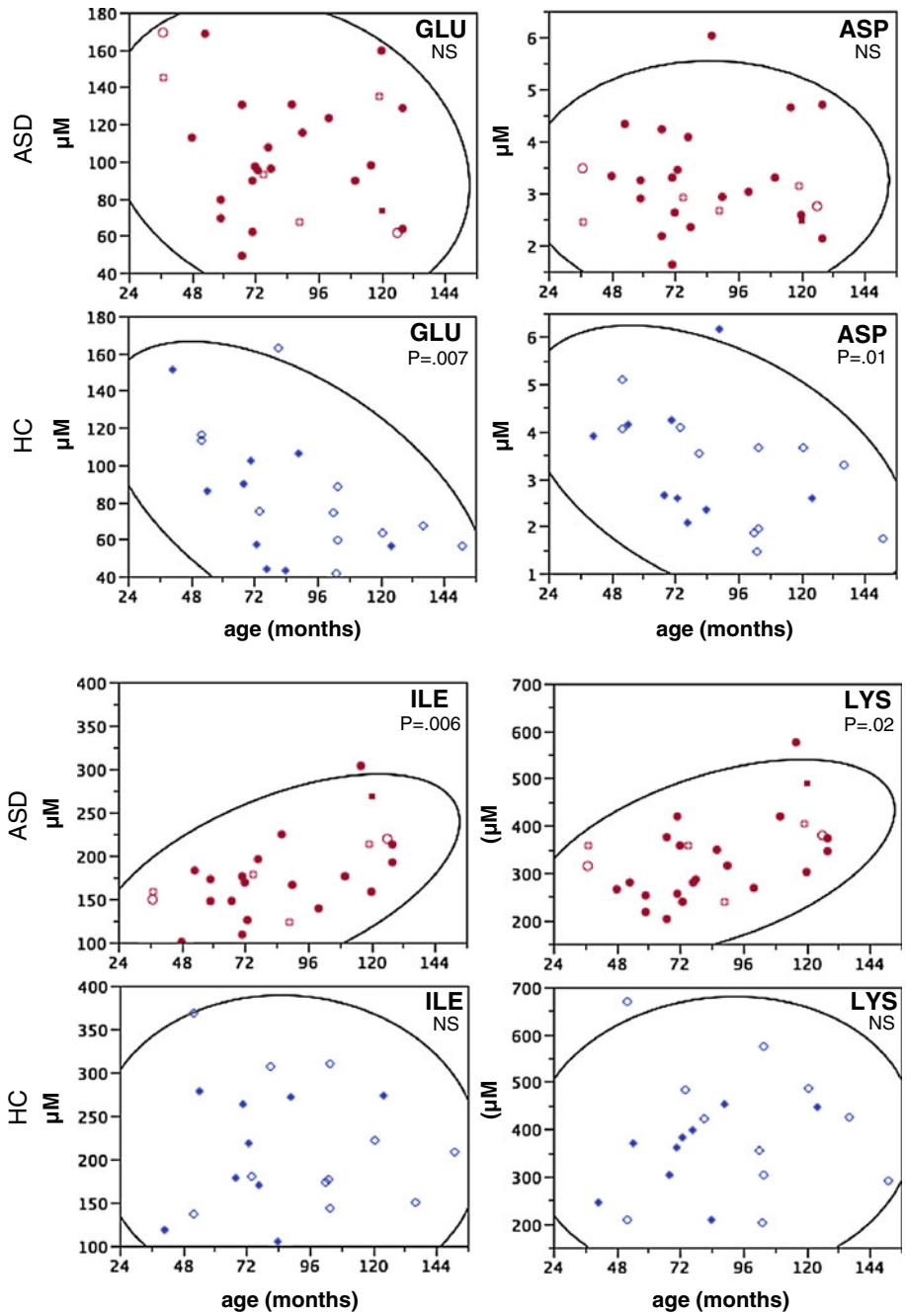

Fig. 2 Distinct developmental regulation of plasma amino acid (AA) levels in children with Autism Spectrum Disorders (ASD). The levels of GLU, ASP, ILE, and LYS are plotted against age for both ASD and healthy control (HC) groups. *Open symbols* represent females and *filled symbols* represent males. *Ellipses* represent the 95% confidence interval for the Spearman correlation test (as appropriate for the non-parametric AA data), with corresponding *p* values as shown. *NS* not significant

healthy controls in adulthood, as previously reported (Shinohe et al. 2006). Longitudinal sampling is necessary to fully bear out this prediction.

While plasma AA levels are potentially helpful in elucidating the neurobiology of neuro-psychiatric disorders, the relationship between plasma and brain AA levels is not fully characterized. The blood–brain transport of AA relies upon a number of transporters with overlapping specificities (Hawkins et al. 2006). This substrate overlap protects the brain from AA deficiencies. The levels of AA are normally lower in the cerebrospinal fluid (CSF) compared to plasma levels, with CSF/plasma ratios of some AA being affected by age, gender, and certain medications (Scholl-Burgi et al. 2008). Although the blood–brain AA transport capacity cannot be measured directly in human subjects, altered transport capacity for several AA, including TYR, has been described in fibroblasts taken from children with autism (Fernell et al. 2007). Further studies are needed to determine the cause(s) of the reduction in plasma levels of the majority of polar neutral AA and LEU in children with ASD observed in our study. Altered AA transport mechanisms commonly affecting polar neutral AA, potentially via the AA transporter subunit LAT2 (Hyde et al. 2003), may be considered for investigation.

Table 4 Predictive values of individual and combined amino acid (AA) plasma levels for identification of subjects with Autism Spectrum Disorders (ASD) versus healthy control (HC) subjects

Predictor	Area under the ROC curve	p
Individual AA		
THR	.80	.0004
GLN	.87	$<10^{-4}$
Combinations of AA		
GLN × GLU	.89	$<10^{-4}$
GLN × LEU	.89	$<10^{-4}$
GLN × TYR	.92	$<10^{-4}$
GLN × LEU × GLU	.92	$<10^{-4}$
GLN × TYR × LEU × GLU	.92	$<10^{-4}$
GLN × TYR × ASN	.96	$<10^{-4}$

Indicated are predictors with good to excellent predictive value (area under the receiver operating characteristic (ROC) curve of .80 or above), in order of increasing area under the ROC curve

Dietary intake and absorption may also influence plasma AA levels. Peculiar dietary habits (Ahearn et al. 2001; Schreck et al. 2004), diet selectivity (Arnold et al. 2003), functional GI abnormalities (Adams et al. 2011), and altered intestinal bacterial microflora (Finegold et al. 2010) have been observed in children with ASD. These factors may contribute to altered levels of plasma AA in ASD. In this study, we have not accounted for fasting as well as previous day and overall diets, or for the presence of any GI symptoms, which future studies will need to address.

In search of an AA profile that could discriminate ASD patients from healthy controls, we found that specific AA, alone or in combination with each other, yielded very good to excellent predictive values. The best predictive value was .96, for the combinations of plasma levels of GLN, TYR, and ASN, all of which are reduced in plasma of ASD patients. Further studies are necessary to confirm this preliminary finding of the predictive ability of plasma AA measurements in discriminating children with ASD.

In this study, we found that some AA levels were age-dependent in ASD and HC groups, following group-specific trends, notably GLU, ASP, ILE, and LYS. We did not find any effect of gender, ethnicity, time of blood draw, medications, cognitive function on AA levels within each group, or of the diagnosis (i.e., autism vs. PDD-NOS)

Table 5 Comparison of our findings with published studies on amino acid levels in subjects with autism/Autism Spectrum Disorders (ASD)

	Moreno-Fuenmayor et al. (1996)	Aldred et al. (2003)	Shinohe et al. (2006)	Our study
Biological fluid	Plasma (not specified)	Platelet-rich plasma	Serum	Platelet-poor plasma
Patient cohort:	AU (N = 14)	AU & SIB (N = 36)	AU (N = 18)	ASD (N = 27)
Control cohort:	None (external reference)	None (external reference)	Healthy (N = 19)	Healthy (N = 20)
Age range:	<10 years old	4–30 years old	18–26 years old	3–11 years old
AA detection method:	HPLC	HPLC	HPLC	LC–MS
Statistical test:	Unpaired t test	Wilcoxon rank sum	Unpaired t test	Wilcoxon rank sum
Number of simultaneous AA measures:	5 reported	17	5	20
Bonferroni correction	Not applied	Not applied	Applied	Applied
Summary of results:	AU < external ref: GLN, ASN ($p < .01$)	AU & SIB < external ref: GLN ($p < .05$)		ASD < CON: GLN, THR, SER, ASN, CIT, TYR, LEU ($p < .025$)
				ASD < CON: PHE, TRP, HIS, MET, ILE ($p < .05$)
			AU = CON: GLN, GLY, SER	ASD = CON: LYS, VAL, ARG, PRO, GLY, ALA, ASP
	AU > external reference: GLU, ASP (for some patients)	AU & SIB > external reference: GLU, PHE, ASN, TYR, ALA, LYS ($p < .05$)	AU > CON: GLU ($p < .001$)	ASD > CON: GLU ($p < .02$)

AA amino acids, *ASD* subjects with Autism Spectrum Disorders, *AU* subjects with autism, *CON* control subjects, *HPLC* high performance liquid chromatography, *LC–MS* liquid chromatography mass spectrometry, *SIB* unaffected siblings

within the ASD group. Furthermore, we did not find any associations between clinical scores, namely ADI-R and ADOS, and any AA levels in the ASD group. However, this study bears several limitations, including relatively small and uneven group sizes, lack of matching by gender, imprecise matching for ethnicity and age (although not different statistically), and the absence of a disease control group.

To summarize our findings, children with ASD in our study had altered plasma levels of most polar neutral AA and LEU. The cross-sectional developmental pattern of several AA levels was also different in the ASD group compared to the HC group. These findings warrant further examination of plasma AA levels in larger groups of children with ASD while assessing for any relationships with clinical features, ideally in serial longitudinal analyses. Finally, our study, while preliminary, demonstrated the potential for developing an AA-based biological profile that might help with better characterization of individuals with ASD.

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