



Allergic disease and low ASQ communication score in children

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ABSTRACT

Autism Spectrum Disorders (ASD) are complex and multifactorial. Previous investigations have revealed associations between allergic disease and ASD, which are characterized by impaired communication skills. In this study we observed an association between allergic disease and communication skills development as assessed by the Ages and Stages Questionnaire (ASQ) communication score, as a proxy for ASD, among children who participated in the Vitamin D Antenatal Asthma Reduction Trial (VDAART). In particular, we observed significant associations between both a diagnosis of eczema at age 3 years (OR = 1.87; confidence interval [CI]: 0.97–3.47; $p = 0.054$) and a diagnosis of food allergy at age 6 years (OR = 3.61; 95% CI: 1.18–9.85; $p = 0.015$) with ASQ communication score. Plasma metabolomics analyses suggest that dysregulated tryptophan metabolism may contribute to the pathogenesis of these co-morbidities.

1. Introduction

Autism spectrum disorder (ASD) is a complex and heterogeneous developmental disorder characterized by difficulty with communication and interaction, spanning various social contexts (Masi et al., 2017). The etiology of this condition is still being explored, however prior evidence points to a multifactorial etiopathogenesis resulting from complex interactions between genetic (Li et al., 2012; Liu and Takumi, 2014) and environmental factors (Posar and Visconti, 2017). These environmental factors are hypothesized to underlie the increasing prevalence of ASD; which is estimated to be as high as one in every 59 people in the United States, and between 1% and 2% globally (Data & Statistics, 2019). Interestingly, there is a growing body of evidence linking ASD to allergic disease (Xu et al., 2018; Lyall et al., 2015; Miyazaki, 2015); the prevalence of which has also increased over the last few decades (Thomsen, 2015). However, the mechanisms underlying this purported relationship are not fully elucidated (Miyazaki, 2015).

The Ages and Stages Questionnaire (ASQ) is a widely used tool for assessment of a child's early development. The ASQ surveys five developmental skill domains: communication, gross-motor, fine-motor, problem solving, and personal-social. The communication domain has been used as a proxy measure to identify children who are at a higher

risk of a later ASD diagnosis (Hardy et al., 2015; Kelly et al., 2019; Øien et al., 2018). We previously observed a significant relationship between a low ASQ communication score at age 3 with a subsequent diagnosis of ASD among children from the Vitamin D Antenatal Asthma Reduction Trial (VDAART) (Kelly et al., 2019). Our results suggested that dysregulated tryptophan metabolism may play an important role in delayed development of communication skills and therefore potentially in ASD diagnosis. Tryptophan metabolism has also been implicated in the pathophysiology of allergic disorders (Gostner et al., 2016). Therefore, in the current study, we investigated the relationship between the ASQ communication score and allergic disease in children from VDAART (Litonjua et al., 2014) with a specific focus on tryptophan metabolism.

2. Methods

2.1. Study population

This study was nested within the VDAART; the details of which have been published previously (Litonjua et al., 2014). In brief, pregnant, women with a history of allergic disease who were between 10 and 18 weeks of gestation were recruited and randomized 1:1 to a daily dose of vitamin D or a placebo and their offspring followed-up. This trial was approved by the IRBs of the participating institutions and all

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participants provided written consent at the time of enrollment.

2.2. Data collection

VDAART included eczema and food allergy as secondary outcomes. Atopic eczema was defined as parental report of physician diagnosed eczema, a persistent rash and the presence of sensitization to at least one allergen or a total serum IgE above the geometric mean value (Litonjua et al., 2014). Food allergy was defined by a parent-reported allergic reaction to a food by a given age with serum specific IgE to that food ≥ 0.35 kU/l. We used data based on the ages 3 and 6 follow-ups. Asthma and recurrent wheeze were defined by a self-report of a doctor's diagnosis and wheezing as described previously (Litonjua et al., 2016). The children in VDAART are now between 6 and 8.5 years old, and study follow up is ongoing.

2.3. ASQ

The ASQ 3rd Edition was administered to the primary caregivers of the VDAART children at their age one, two and three year visits. In this analysis we used data from the age 3 ASQ. The questionnaire included five developmental domains for assessment: gross motor skills, fine motor skills, problem solving ability, personal/social skills, and communication. For each domain, based on their score children are categorized as: (i) "On Schedule for developing normally" (above the mean); (ii) "Requires Monitoring" (1–2 standard deviations below the mean); (iii) "Needs further evaluation" (> 2 standard deviations below the mean).

2.4. Tryptophan metabolism

A subset of children had metabolomic profiling available on plasma collected at age 3 years. Plasma sample profiling was performed using ultrahigh performance liquid chromatography-tandem mass spectroscopy (LC/MS) by Metabolon Inc, as described previously (Blighe et al., 2017; Kelly et al., 2018). Based on our previous findings (Kelly et al., 2019) we focused on the 15 available plasma metabolites in the tryptophan metabolism sub-pathway (as defined by Metabolon Inc).

2.5. Statistical analyses

ASQ communication score at age 3 was our outcome of interest, concurrent to the age three metabolomic profiling. We used a binary cut-off for communication score comparing those "On schedule for developing normally" to those either requiring "further evaluation" or "monitoring", based on the recommendations of Hardy et al. (2015) and our previous work, in which we identified a significant relationship between this metric and future development of ASD (Kelly et al., 2019). We define the combination of the lower two categories as a 'low communication score'. We used a chi squared test of independence and logistic regression models adjusting for race, gender and study site to investigate differences in baseline characteristics and in the frequency of allergic conditions (asthma, food allergy and eczema at ages 3 and 6) between children classified as "on schedule" as compared "low communication score". VDAART was a randomized double-blinded trial which supplemented mothers with vitamin D or a placebo throughout pregnancy (Litonjua et al., 2014), therefore we additionally ran our analyses stratifying by intervention arm. Similarly, given the known sex disparity in ASD (Masi et al., 2017), we further ran a sex-stratified analysis.

We then explored whether disease burden at age 3 or 6 years was associated with greater dysregulation in the tryptophan pathway, using analysis of variance (ANOVA) to look for differences in the levels of 15 tryptophan metabolites across 4 groups: 'control' (no relevant condition, $n = 252$); '1 condition' (low communication score, $n = 22$); '2 conditions' (low communication score and either food allergy or

eczema, $n = 12$) or '3 conditions' (children with eczema, food allergy and a low communication score, $n = 3$). Subjects with an "on schedule" ASQ communication score and food allergy or eczema were excluded ($n = 114$).

3. Results

3.1. Study population

Of 806 children in VDAART, 715 (88.7%) had completed ASQ questionnaires at age 3. Based on their communication score; 652 (91.2%) were classified as "On Schedule" (score > 41.44), sixty-three (8.81%) had a low communication score: 42 (5.87%) "required monitoring" (score 30.99–41.44) and 21 (2.94%) "needed further evaluation" (scores < 30.99) (Supplemental Fig. 1).

Children in the low communication score group were more likely to be diagnosed with ASD by year 8 (11.1% vs 1.2%; $p < 0.001$) (Supplemental Table 1). Children with low communication scores were also less likely to be from San Diego than Boston or St. Louis (17.5% in San Diego, 41.3% in St. Louis, 41.3% in Boston; $p = 0.012$), and more likely to be male (68.3% vs 51.7% male; $p = 0.017$). There were no major differences by racial group or vitamin D treatment group.

3.2. ASQ-3 and allergic conditions

Children in the low communication score group were more likely to report food allergy (Table 1). This association was significant for children with food allergy at age 6 (17.6% in the low score group vs. 4.7% on-schedule group; $p = 0.007$) and for those with food allergies at either age 3 or age 6 (13.5% vs 5.0%; $p = 0.029$). After adjustment for site, gender, and race, we observed that children diagnosed with food allergy at either age 3 or 6 were almost three times more likely to be in the lowest ASQ-3 communication score categories (OR = 2.77; 95% CI: 1.03–6.64; $p = 0.030$) (Table 1). This association appeared to be driven by the age 6 diagnoses (OR = 3.62; 95% CI: 1.19–9.86; $p = 0.015$). There were also more children with eczema at age 3 in the low communication score group (27.0% vs 16.0% $p = 0.040$), and after adjustment for confounders, we found that children diagnosed with eczema at age 3 had almost double the risk of being in low communication score category, compared to children without eczema (OR = 1.87; CI(95%): 0.97, 3.47; $p = 0.054$). However, this association was not evident based on age 6 eczema diagnosis. Children with a low communication score at age three were also more likely to report a diagnosis of asthma at both age 3 and age 6, but these associations did not reach significance.

3.3. Sensitivity analyses

There was evidence that the ASQ-3 communication score – allergic disease relationship was stronger in those children whose mothers did not receive vitamin D supplementation during pregnancy (Supplemental Table 2). This was particularly evident for the association between ASQ communication score and food allergy at age 6; OR 10.15 (95% CI 2.09, 47.14) $p = 0.003$ in those whose mothers received placebo during pregnancy, compared to OR 1.70 (95% CI 0.24, 7.43) $p = 0.525$ in those whose mothers received vitamin D. Conversely, there was no evidence of a sex-specific effect, and an interaction analyses was non-significant ($p > 0.05$) for all investigated variables (results not shown).

3.4. Tryptophan metabolism

The subgroup of children with metabolomic profiling were representative of the parent VDAART population in terms of race, gender, and treatment group, however, there was a slightly higher percentage of children from St. Louis (44.7% vs 36.2%, $p = 0.002$ Supplemental

Table 1
ASQ communication score at age 3 and the association with food allergy, eczema and asthma.

	ASQ Communication Score Category at Age 3					
	On Schedule n(%)	Low Communication Score n(%)	Chi Square P-Value	Odds Ratio	Confidence Interval	Logistic Regression P-Value
Food Allergy						
Year 3						
No	449(96.1)	45(91.8)				
Yes	18(3.9)	4(8.2)	0.294	1.94	0.53,5.71	0.264
Year 6						
No	342(95.3)	28(82.4)				
Yes	17(4.70)	6(17.6)	0.007	3.62	1.18,9.85	0.015
Year 3 or 6						
No	495(95.0)	45(86.5)				
Yes	26(5.0)	7(13.5)	0.029	2.77	1.03,6.63	0.030
Eczema						
Year 3						
No	548(84.0)	46(73.0)				
Yes	104(16.0)	17(27.0)	0.040	1.87	0.97, 3.47	0.054
Year 6						
No	520(88.6)	52(89.7)				
Yes	67(11.4)	6(10.3)	0.978	0.807	0.29,1.88	0.645
Year 3 or 6						
No	459(78.2)	41(70.7)				
Yes	128(21.8)	17(29.3)	0.254	1.33	0.69,2.48	0.384
Asthma						
Year 3						
No	478(73.4)	39(61.9)				
Yes	173(26.6)	24(38.1)	0.071	1.45	0.82,2.50	0.191
Year 6						
No	360(55.2)	28(44.4)				
Yes	292(44.8)	35(55.6)	0.132	1.37	0.80,2.34	0.249
Year 3 or 6						
No	358(55.0)	28(44.4)				
Yes	293(45.0)	35(55.6)	0.141	1.36	0.80,2.32	0.261

Data from 199 children missing for food allergy at age 3, 322 missing for food allergy at age 6, 142 missing for food allergy at age 3 or 6.

Data from 70 children missing for eczema at age 6, 70 missing for eczema at age 3 or 6.

Data from 1 child missing for asthma at age 3 and 1 missing for asthma at age 3 or 6.

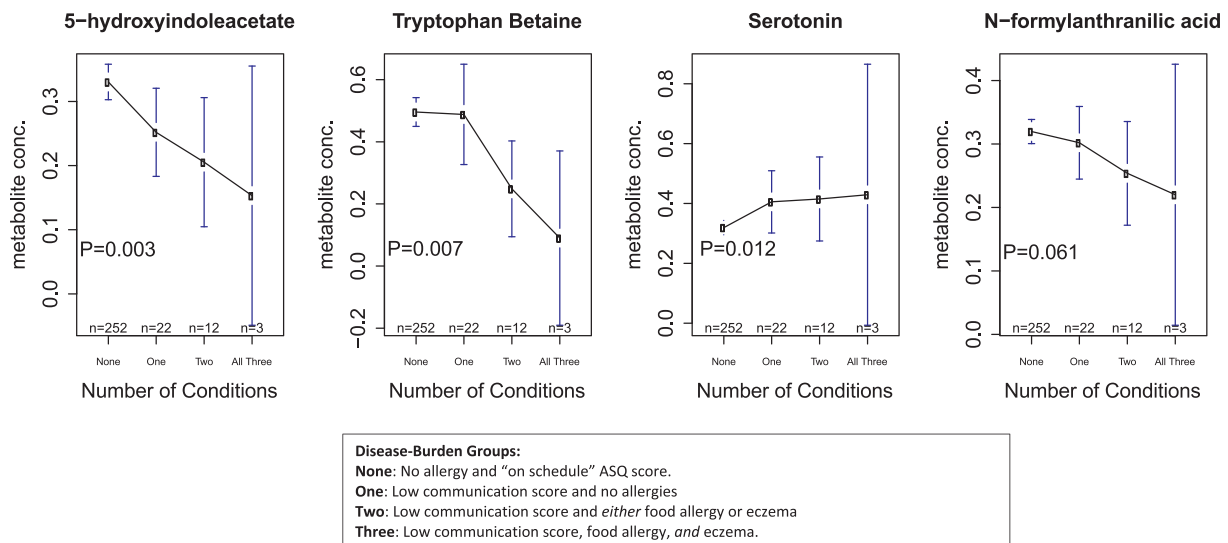


Fig. 1. Age 3 Year Metabolite Concentrations Across Disease-Burden Group.

Table 3). 5-hydroxyindoleacetate, tryptophan betaine, serotonin, and N-formylanthranilic acid were all significantly different across disease-burden groups. 5-hydroxyindoleacetate, N-formylanthranilic acid and tryptophan betaine levels decreased with increasing disease burden; and were lowest in subjects with all three conditions. The opposite trend emerged for serotonin, with higher levels of this metabolite in children with all three conditions and decreasing progressively with

lower disease burden (**Fig. 1**).

4. Discussion

The reported prevalence of both ASD and allergic disorders continue to rise globally (**Thomsen, 2015**); and these diseases often co-occur, suggesting a common etiology (**Xu et al., 2018; Lyall et al., 2015**). In

this study, we investigated the relationship between poor early life communication development, as assessed by the ASQ-3 communication score, and allergic disease, with the hypothesis that the ASQ communication score is a good proxy for early detection of ASD. In order to explore potential biochemical modulators of this relationship, we leveraged plasma metabolomic data on a subset of these children with a focus on tryptophan metabolism.

In this analysis, we observed that children classified in the lowest two categories of communications skills development at age three were more likely to be diagnosed with food allergy or eczema, an association that persisted after adjustment for study site, race, and gender. This finding is in agreement with a growing body of literature reporting positive associations between allergy and ASD (Xu et al., 2018; Lyall et al., 2015).

There is a strong biological rationale for a link between allergic disorders and ASQ. Although the etiology of ASD is largely unknown, it is commonly agreed to be multifactorial (Posar and Visconti, 2017), with compelling evidence to suggest that immune dysregulation may play a role. For example, subjects with ASD often display neuro-inflammation in brain tissue (Zerbo et al., 2015; Vargas et al., 2005). Given the well-established relevance of immunologic perturbations to allergic conditions (Kelly et al., 2018); it is possible that there are shared biological mechanisms linking these conditions (Xu et al., 2018; Lyall et al., 2015). Recent literature suggests that an ASD diagnosis may render an individual more susceptible to allergic disease, potentially relating to the activation of mast cells that accompany atopy (Theoharides et al., 2016). Studies have also reported higher levels of Th2 cytokines, elevated serum IgE, and eosinophils in the blood of subjects with autism lending greater plausibility to the hypothesized relationship between ASDs and allergic response (Xu et al., 2018; Khakzad et al., 2012).

In previous work, we determined that tryptophan metabolism may be involved in communication skills development, which is in agreement with recent research into abnormal tryptophan levels and ASD (Kałużna-Czaplińska et al., 2017). Intriguingly, the tryptophan pathway has also been identified as relevant to the pathophysiology of allergic disease, with alterations in tryptophan metabolism reported in patients suffering from allergy and eczema (Gostner et al., 2016; Ito et al., 2004). Therefore, we examined tryptophan metabolites as a possible common biochemical link between allergy and ASD risk, focusing on a burden of disease, classifying subjects according to ASQ score and number of allergic conditions. Three metabolites in the tryptophan pathway significantly varied across subjects with different burdens of disease, and one was borderline significant. Levels of serotonin increased, while tryptophan betaine and 5-hydroxyindolacetate, and N-formylanthranilic acid decreased with increasing burdens.

Higher plasma serotonin in subjects with ASD as well as allergy has also been observed (Muller et al., 2016; Ciprandi et al., 2011). In the plasma of children with ASD, the tryptophan biosynthesis pathway may be biased towards the production of serotonin over other downstream products (Chugani et al., 1999). Serotonin is involved in multiple aspects of neurological function that may influence the development of ASD such as regulation of mood, sleeping and appetite patterns, and social interaction (Muller et al., 2016). Increased serotonin levels have also been linked to allergic disease, with reports that human mast cells may synthesize and release serotonin, thus illuminating a potential link to allergic response (Ciprandi et al., 2011; MacHaffie et al., 1960). This may explain why we also identified other metabolites downstream of serotonin catabolism, such as 5-hydroxyindolacetate, as significant.

Our study generated compelling results linking allergic disease to delayed communication skills development and provides evidence to support dysregulated tryptophan metabolism as a possible common etiological factor. However, there were a number of limitations. In this study, age 3 ASQ communication score served as a proxy for ASD, which we were underpowered to explore in this cohort, with only 15 cases. The use of the ASQ communication score as a proxy is based on

evidence from the literature (Hardy et al., 2015; Øien et al., 2018), however we note that the evidence remains limited and we emphasize that ASDs are complex and multifactorial potentially affecting many other developmental domains. As such we caution that our results pertain to ASQ assessed communication skills and extrapolation to ASD is exploratory.

Even when considering ASQ low-communication score, we were limited by sample size particularly for the tryptophan analysis; there were only three subjects in our highest disease burden condition resulting in large confidence intervals. In contrast, within the confines of the existing VDAART dataset we were only able to assess IgE mediated food allergy. A proportion of food allergy is non IgE mediated and therefore our numbers for food allergy are likely to be an underestimate. Nevertheless, we believe that these results generate biologically meaningful hypotheses for the improved understanding of these complex conditions. Further studies in larger cohorts with more children in the lowest ASQ communication score categories, and which additionally consider other developmental domains are warranted.

It has been suggested that distress due to the irritation and discomfort experienced as a result of allergic disease may exacerbate the behavioral symptoms of ASD and communication behavior (Hughes et al., 2018). However, due to the cross-sectional nature of this study, we were not able to establish causality in terms of direction of effect between allergic disease and communication skills development. Rather, we hypothesize that these conditions all share a common underlying etiology, potentially involving epigenetic changes *in utero*. Maternal health during pregnancy, especially during critical windows of development, has been shown to influence the health and behavior of offspring (Ciernia and LaSalle, 2016). Disruption to the mother's immune systems, and in particular maternal allergic asthma, have been shown to increase the risk of ASDs and other developmental neurological disorders in their children (Knuesel et al., 2014). Similarly, maternal history of immunological disorders has been shown to predict the risk of asthma, allergy and eczema (Bin and Leung, 2016; Ohya et al., 2005). It is suggested these effects may be mediated through epigenetic changes passed down from mother to child as evidenced by transcriptomic dysregulation of common pathways, including those involved in immune function (Ciernia and LaSalle, 2016; Schwartz et al., 2015). Intriguingly, serotonin synthesis, which is particularly sensitive to perturbations to the immune system during development, has been proposed to be central to this link (Schwartz et al., 2015).

There were also significant differences in ASQ communication score across study sites. In our San Diego site, which had the highest socioeconomic status of the sites there were no subjects in the lowest category, which may reflect unmeasured biases related to parental reports of child development. We controlled for study site in our analysis to account for this discrepancy and determined that our results for food allergy and eczema retained significance after adjustment for study site and other potential confounders.

The significance of our results is two-fold. Firstly, this study adds to the small but growing body of literature on allergy and communication skills development in early life. Secondly, we propose a biological basis for the observed comorbidity of allergy and ASD using a metabolomic analysis of plasma. Allergic disease is one of the most common chronic health conditions globally (Jackson et al., 2013); and determining a common etiological pathway, which may relate to dysregulated tryptophan metabolism, with ASD could have important implications for both disorders. Interestingly, vitamin D has been suggested as protective for both neurological development, specifically ASD (Cannell, 2017), and for allergic disease (Mirzakhani et al., 2015). We observed that the relationship between ASD and allergic disease was strongest among VDAART children whose mothers were not supplemented with vitamin D during their pregnancy, suggesting that the common pathology underlying these disorders could potentially be ameliorated with vitamin D supplementation.

These preliminary findings provide compelling hypothesis that are

worthy of future research and more in-depth biochemical analyses in larger cohorts with well characterized neurological data and ASD diagnoses.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbi.2019.10.008>.

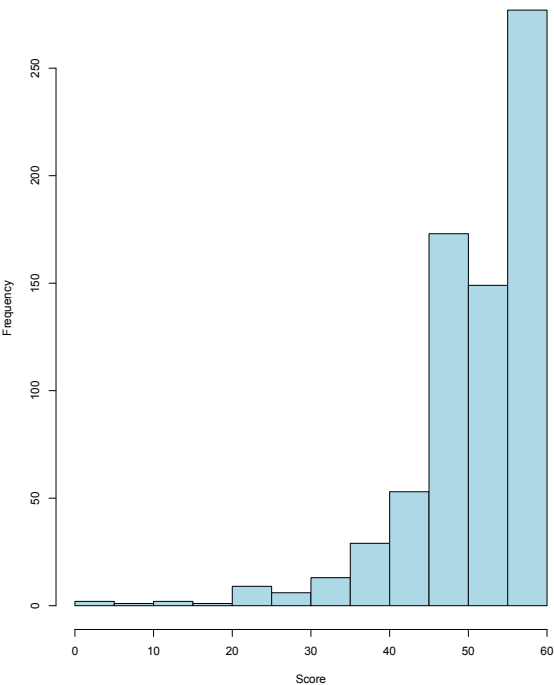
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Allergic Disease and low ASQ Communication Score in Children

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Supplemental Figure 1: ASQ Communication Score Distribution



Communication Score Category	Range	N	%
On Schedule	>41.44	652	91.2
Needs Monitoring	30.99-41.44	42	5.87
Further Evaluation	<=30.00	21	2.94

**Supplemental Table 1: Baseline characteristics for children from the VDAART with ASQ score
at Age 3 Years**

	Developing Normally	Low Score	P-Value
N	652	63	
Autism = Yes (%)	8 (1.2)	7 (11.1)	<0.001
Gender = male (%)	337 (51.7)	43 (68.3)	0.017
Site (%)			0.012
San Diego	217 (33.3)	11 (17.5)	
Boston	174 (26.7)	26 (41.3)	
St. Louis	261 (40.0)	26 (41.3)	
Asthma/Wheeze (%)	173 (26.6)	24 (38.1)	0.071
Race (%)			0.426
Black, African American	314 (48.7)	36 (57.1)	
Caucasian	217 (33.6)	17 (27.0)	
Other	114 (17.7)	10 (15.9)	
Treatment = Yes (%)	328(50.3)	33(52.4)	0.855

Supplemental Table 2: ASQ Communication Score at Age 3 and the association with Food Allergy, Eczema and Asthma stratified by VDAART intervention arm

Vitamin D Arm						Placebo Arm				
	On Schedule	Requires further monitoring/evaluation	Odds Ratio	Confidence Interval	P-Value	On Schedule	Requires further monitoring/evaluation	Odds Ratio	Confidence Interval	P-Value
Food Allergy										
Year 3										
No	231 (96.7)	24 (92.3)				218 (95.6)	21 (91.3)			
Yes	8 (3.3)	2 (7.7)	1.95	0.27,8.86	0.428	10 (4.4)	2 (8.7)	1.96	0.28,8.79	0.424
Year 6										
No	170 (93.9)	17 (89.5)				172 (96.6)	11 (73.3)			
Yes	11 (6.1)	2 (10.5)	1.7	0.24,7.43	0.525	6 (3.4)	4 (26.7)	10.15	2.09,47.14	0.003
Year 3 or 6										
No	254 (94.8)	25 (89.3)				241 (95.3)	20 (83.3)			
Yes	14 (5.2)	3 (10.7)	2.03	0.44,6.96	0.3	12 (4.7)	4 (16.7)	3.82	1.042,13.965	0.043
Eczema										
Year 3										
No	272 (82.9)	24 (72.7)				276 (85.2)	22 (73.3)			
Yes	56 (17.1)	9 (27.3)	1.95	0.77,4.67	0.141	48 (14.8)	8 (26.7)	1.86	0.71,4.54	0.185
Year 6										
No	264 (88.9)	27 (90.0)				256 (88.3)	25 (89.3)			
Yes	33 (11.1)	3 (10.0)	0.969	0.21,3.21	0.963	34 (11.7)	3 (10.7)	0.762	0.17,2.44	0.678
Year 3 or 6										
No	228 (76.8)	21 (70.0)				231 (79.7)	20 (71.4)			
Yes	69 (23.2)	9 (30.0)	1.45	0.57,3.54	0.423	59 (20.3)	8 (28.6)	1.29	0.49,3.14	0.583
Asthma										
Year 3										
No	249 (75.9)	23 (69.7)				229 (70.9)	16 (53.3)			
Yes	79 (24.1)	10 (30.3)	1.12	0.48,2.47	0.779	94 (29.1)	14 (46.7)	2.05	0.92,4.56	0.076
Year 6										
No	185 (56.4)	17 (51.5)				175 (54.0)	11 (36.7)			
Yes	143 (43.6)	16 (48.5)	1.12	0.53,2.36	0.756	149 (46.0)	19 (63.3)	1.81	0.83,4.13	0.144
Year 3 or 6										
No	185 (56.4)	17 (51.5)				173 (53.6)	11 (36.7)			
Yes	143 (43.6)	16 (48.5)	1.12	0.53,2.36	0.756	150 (46.4)	19 (63.3)	1.77	0.81,4.05	0.16

Supplemental Table 3: Comparison of Metabolomics Sub-Cohort with Parent VDAART Cohort

	All VDAART Children	Metabolomics Cohort	P-Value
N	806	403	
Autism=Yes (%)	18 (2.2)	9 (2.2)	1
Gender = Male (%)	421 (52.2)	215 (53.3)	0.76
Site (%)			0.002
San Diego	274 (34.0)	137 (34.0)	
Boston	240 (29.8)	86 (21.3)	
St. Louis	292 (36.2)	180 (44.7)	
Asthma/Wheeze = Yes (%)	218 (29.1)	104 (25.8)	0.257
Race (%)			0.998
Black, African American	390 (48.4)	195 (48.4)	
Caucasian	265 (32.9)	133 (33.0)	
Other	151 (18.7)	75 (18.6)	
Treatment = Yes (%)	405 (50.2)	205 (50.9)	0.887