Medical Sciences

Gluten-free/Casein-free Diet Improves Behavior in a Subset of Children with an Autism Spectrum Disorder

Jeffrey M. Kornitzer[1], Deborah Horenstein[1], Keith W. Pecor[2], and Xue Ming[1]

[1]Division of Child Neurology, Department of Neurology, New Jersey Medical School, Rutgers – The State University of New Jersey, Newark, New Jersey, USA. [2]Department of Biology, The College of New Jersey (TCNJ), Ewing, New Jersey, USA

This study investigated whether a gluten-free/casein-free (GFCF) diet was associated with amelioration of behavioral symptoms, language, and gastrointestinal symptoms in children with autism spectrum disorders (ASDs). A retrospective chart review compared gastrointestinal, developmental, and behavioral symptoms in children with ASDs and gastrointestinal complaints on a GFCF diet to those not on a GFCF diet. Individuals on the GFCF diet were more likely to have reported improved speech ($p < 0.001$), behavior ($p < 0.001$), and severity of gastrointestinal symptoms ($p = 0.038$) than children in the control group. Particularly noteworthy, all children with regressive ASDs on the GFCF diet reported improvement in behavior and/or language. In this cohort, the GFCF diet was generally well-tolerated and without any serious adverse effects.

Autism spectrum disorders (ASDs) | Gluten-free/casein-free (GFCF) diet | Dietary interventions | Regressive autism | Non-regressive autism

Background

Effective treatment modalities for the autism spectrum disorders (ASDs) remain stubbornly elusive. Although a handful of medications may alleviate psychiatric symptoms associated with ASDs[1-4], no treatments have emerged that can reliably manage other core features of autism, including communication disturbances, lack of sociability, sensory integration irregularities, and stereotyped behavior. One burgeoning area of interest has been dietary interventions. Already common place in the management of other neurologic disorders such as epilepsy, dietary interventions for the ASDs have been explored as an approach that could improve core features of the ASDs as well as the frequent comorbid gastrointestinal symptoms in some children with ASDs. Children with ASDs may suffer from high rates of abdominal pain, chronic constipation, diarrhea, and gastrointestinal reflux[5-13]. A growing body of research showing intrinsic abnormalities in intestinal anatomy[14] and alterations in gut microbiota[15-22] supports the link between ASDs and gastrointestinal disturbances. A cause-and-effect relationship between an altered gut state and the core autistic neuropsychiatric symptoms has even been suggested[23, 24]. Given that children with ASDs may have an increased rate of gastrointestinal ailments, much attention has been given to diet as a modifiable variable for children with ASDs. In fact, therapies involving dietary modifications are the most common form of complementary and alternative medicine (CAM) used by families in the treatment of ASDs; of these diets, the most commonly employed is the gluten-free/casein-free (GFCF) diet[25].

An association between ASDs and autoimmunity provides one theoretical underpinning for the use of the GFCF diet in children with ASDs. There has been mounting evidence that a subset of children with ASDs have antibodies to gliadin and casein[26-29]. One of the two primary components of gluten, the protein gliadin is found in most grains (wheat, barley, spelt, and rye). Casein is a protein that is found in all mammalian milk. A diet that is devoid of both gluten and casein-containing foods, known as the gluten-free/casein-free (GFCF) diet has become one of the most attempted interventions for children with ASDs[30].

Numerous interventional studies have examined the effect of the GFCF diet on children with ASDs. Studies using parental reports have noted improvement in assessed behaviors after implementation of the GFCF diet[31-33]. Among those studies, interval improvement in gastrointestinal symptoms was also reported[32]. Interestingly, one of the studies noted that those with more severe pre-treatment gastrointestinal symptoms had more impressive post-treatment behavioral improvement[33]. Two randomized, controlled studies found that children with ASDs on a GFCF diet showed significant improvements in both behavior and communication[34,35]. At the same time, several studies including one randomized, double blind study did not find any significant improvement in behavior in children with ASDs on a GFCF compared to a placebo group[36, 37]. Due to a lack of large-scale, randomized studies, coupled with the possibility of adverse consequences associated with GFCF diets, several reviews did not find sufficient evidence to uniformly recommend the GFCF diet for all children with ASDs[38-41].

Given the seemingly inconsistent results among these various studies, it is difficult to arrive at a cohesive understanding of the role that the GFCF diet may play in the management of ASDs. It may be that some of the heterogeneity of results stems from the intrinsic heterogeneity of the population. Increasingly, it is recognized that, as the name of the entity itself implies, the ASDs are a spectrum of disorders[42-44]. Given the diversity of the ASDs at a clinical and even pathophysiological level, it may be difficult to find a treatment modality that reliably works within the entire population. One mode to identifying an appropriate treatment modality then becomes to identify characteristics of specific subpopulations within the larger cohort of ASDs that respond to particular treatments. Consistent with this approach, a consensus report by the American Academy of Pediatrics, while finding that a GFCF diet could not be routinely recommended in ASDs, did observe there could be subgroups that do respond to elimination diets[45]. We hypothesized that, in line with previous findings from other groups, there would be an improvement in behavior, language, and gastrointestinal symptoms with use of the GFCF diet. Furthermore, a subgroup within the larger population of children with ASDS would specifically be "responders" to the GFCF diet.

Abbreviations: ASDs – autism spectrum disorders; GFCF – gluten-free/casein-free

* Corresponding Author: Jeffrey M. Kornitzer, MD, Division of Child Neurology, Department of Neurology, New Jersey Medical School, Rutgers – The State University of New Jersey, Suite 5200, Doctor's Office Center, 90 Bergen Street., Newark, NJ 07101-1709, USA. Phone: (973) 972-7151. Email: j.kornitzer@rutgers.edu.

Table 1. Six-point Gastrointestinal Severity Index (6-GSI) scoring system. Up to two points are assigned for each of the six symptoms. A higher 6-GSI score reflects more severe symptoms.

	Score		
	0	1	2
Constipation	≥ 5 stools per week	3-4 stools per week	0-2 stools per week
Diarrhea	0-1 loose stools per day	2-3 loose stools per day	≥ 4 loose stools per day
Average Stool Consistency	Formed	Loose/unformed ≥ 3 days per week	Watery ≥ 3 days per week
Stool Smell	Normal	Abnormal ≥ 3 days per week	Unusually foul ≥ 3 days per week
Flatulence	Normal	Frequent ≥ 3 days per week	Daily
Abdominal pain	None	Mild discomfort ≥ 3 times per week	Moderate to severe discomfort ≥ 3 times per week

Table 2. Subject Age, Gender, Subtype of ASD, Gastrointestinal (GI) Severity Index scores

	Control Group (Not on GFCF Diet)	Intervention Group (On GFCF Diet)
Number of Subjects	45	21
Average Age (years)*	12.6	12.2
Proportion of Males	82% (N=37)	67% (N=14)
Proportion of regressive-type ASDs	9% (N=4)	38% (N=8)
Average Initial GI Severity Index Score	3.0	3.4
Average GI Severity Index Score after GFCF Diet or equivalent time (10 months)	1.5	0.9

* Age at time of final analysis of behavior and language. (GFCF= Gluten-free/ Casein-free; ASDs= Autism spectrum disorders)

Methods

Design

The study was conducted through a retrospective chart review of patients seen by both the Division of Child Neurology and the Division of Pediatric Gastroenterology in Rutgers-New Jersey Medical School in Newark, NJ, USA. On the basis of an extensive history and physical/neurological examination, a Child Neurologist made the diagnosis of an ASD consistent with the clinical definition in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*[46]. Further history was obtained in order to determine whether the patient had consistent developmental delays since birth ("non-regressive") or whether the patient had apparently normal development followed by a regression of milestones such as loss of words previously used persistently, loss of eye contact, loss of gestures, and loss of ability to respond to names ("regressive"). A pediatric gastroenterologist evaluated each subject and assessed the quality and severity of their gastrointestinal symptoms. Upon chart review, this information was quantified using a modified version of the GI Severity Index [Table 1]. This 6-item scale assigns up to two points each for constipation, diarrhea, average stool consistency, stool smell, flatulence, and abdominal pain, with a higher score correlating with more severe symptoms[18, 47]. Based upon parental preference, some of the patients were started on a GFCF diet. Parental reports of behavioral symptoms as well as communication skills both before and after the diet were compared to reports obtained at similar time intervals from children with ASDs and abdominal symptoms not on the GFCF diet.

Study Population

The subjects were children with ASDs ages 8 to 24 years old (mean age 12.5 years; standard deviation 4.7) [Table 2]. With 77.3% of the subjects being male, the gender distribution is consistent with the known male predilection for ASDs, as represented in the approximate 4.7:1 male-to-female ratio of ASDs in New Jersey[48]. The subjects were from the greater Newark, New Jersey area and surrounding neighborhoods; the majority of subjects were insured by Medicaid. Among the subjects of this study, twelve had a regressive-type ASD and the remainder (fifty-four children) had a non-regressive ASD. Only children with an idiopathic ASD, that is an ASD without a clear underlying genetic or other predisposing syndrome, were included in this study. In order to avoid the confounding variable of having overt celiac disease (which necessitates treatment with a gluten-free diet), any child with seropositivity to tissue transglutaminase and/or endomysial antibody was excluded from the study.

This study was approved by the Rutgers-New Jersey Medical School Institutional Review Board. The written consent process was waived because this was a retrospective study.

Data Collection

A retrospective chart review was performed, collecting data on all common patients with a diagnosis of an ASD who were seen by both Child Neurology and Pediatric Gastroenterology prior to the onset of data collection and for at least the extent of the studied time interval. After collecting demographic data, patients were stratified into two groups: intervention (that is, on a GFCF diet) and control (that is, not on a GFCF diet). Results of behavioral and language assessments by Child Neurologists were used to establish pre-intervention and post-intervention measures for those on the GFCF diet. Differences in those assessments were used to compare improvement in the control group over the same time frame of six to twelve months (mean of 9.5 months). Improvements in behavior were defined as less frequent stereotyped behavior or less frequent aggressive behavior. Improvement in language was defined as an increase in the number of expressed words. Based upon information gleaned from the Pediatric Gastroenterologist, a score for the GI Severity Index was assigned for the end point, too.

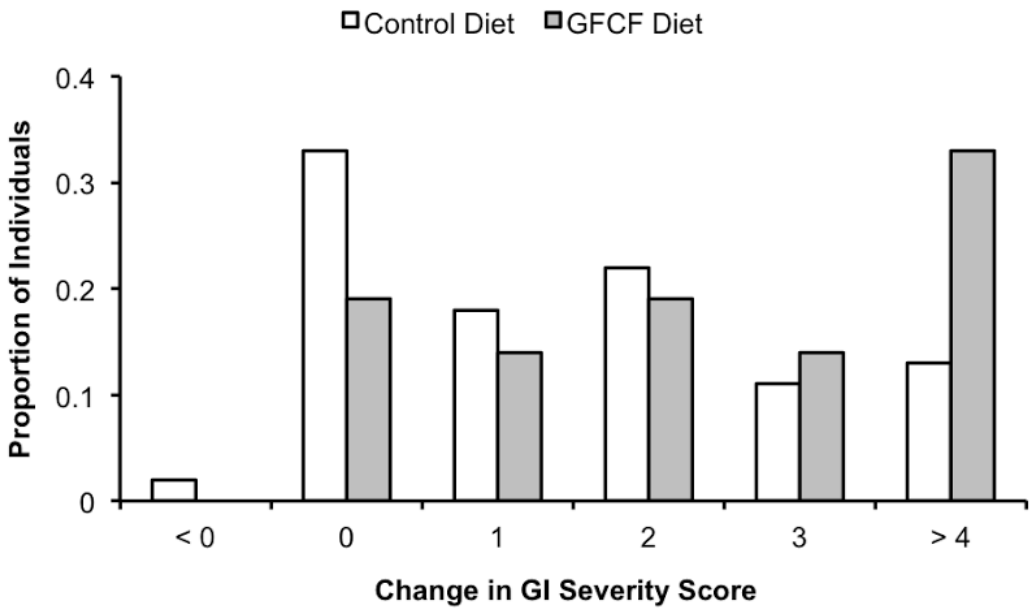

Figure 1. Change in Gastrointestinal (GI) Severity Index score by group. The change in GI Severity Index score is calculated as pre-treatment score minus post-treatment score. Therefore, a higher change in GI Severity Index score reflects a greater relative improvement in GI symptoms as measured by the scale. (GFCF = Gluten-free/Casein-free).

Data Analysis

In evaluating gastrointestinal (GI) severity, the score after the conclusion of the experimental diet or control period was subtracted from the score before the inception of the diet / control to provide a measure of change. As such, a negative change in GI severity would indicate a worsening of symptoms, and a positive change would indicate a lessening of symptoms. The data for GI severity changes were ranked and contrasted using a Mann-Whitney U test. For speech and behavior, observed and expected values for improvement and absence of improvement were contrasted between diet treatments using contingency tables. All statistical calculations were made using SPSS Statistics v21-23.

Results

Tolerability

There were a total of 66 patients for whom we had follow-up information. Of those 66 patients, 21 patients tried the GFCF diet. Patients were maintained on the diet, on average, for 9.5 months (Standard Deviation = 7.97 months). The most common reasons for discontinuing the diet were that it was too difficult to adhere to or that no improvement on the diet was noted. Parents of children on the GFCF diet did not note worsening gastrointestinal symptoms or weight loss. In addition, there was no worsening of either language or behavior in any patient on the GFCF diet, as compared to the control group in which 18% of the patients received reports of worsening behavior and/or language over the same time frame.

Gastrointestinal Symptoms

GI Severity Index score reduction was significantly greater in the experimental diet treatment than in the control treatment ($U = 325$, $p = 0.038$) [Figure 1]. Of particular note, 33% of those individuals on the experimental diet saw a reduction in GI severity symptoms of four or more.

Behavior and Language

Of the twenty-one patients on the GFCF diet, sixteen patients (76%) had improvement in language, behavior, or both. Of the forty-five patients not on a dietary intervention, only one patient was reported to have had improvement in behavioral symptoms and this was related to toileting behavior. Eight patients out of those forty-five patients (18%) actually had worsening of their autistic symptoms, specifically of behavior.

Individuals on the experimental diet were more likely than individuals in the control treatment to receive reports of improved speech ($\chi^2 = 22.33$, $df = 1$, $p < 0.001$) and improved behavior ($\chi^2 = 30.52$, $df = 1$, $p < 0.001$). No individuals in the control treatment experienced improvement in speech, whereas 43% of individuals in the diet treatment experienced improvement in speech. Similarly, only 2% of individuals in the control treatment experienced improvement in behavior, compared to 62% of individuals on the experimental diet.

Although the study numbers were too small for subgroup analysis, it is interesting to note that of the twenty-one patients on the GFCF diet, eight patients had a regressive-type ASD [Figure 2]. Interestingly, all eight of these patients had improvement in at least one domain: two patients showed improvement in language only, one patient showed improvement in behavior only, and five patients showed improvement in both language and behavior. Of the forty-five patients not on a dietary intervention, four had a regressive-type ASD. None of these four patients had improvement and one actually had worsening behavior.

Discussion

In concert with our hypothesis, parents reported that children with ASDs on the GFCF were significantly more likely to have improvements in behavior, language, and gastrointestinal symptoms. In addition, there seemed to be tolerability to the diet. These findings are similar to previously published studies that have noted improvement in measured behaviors after

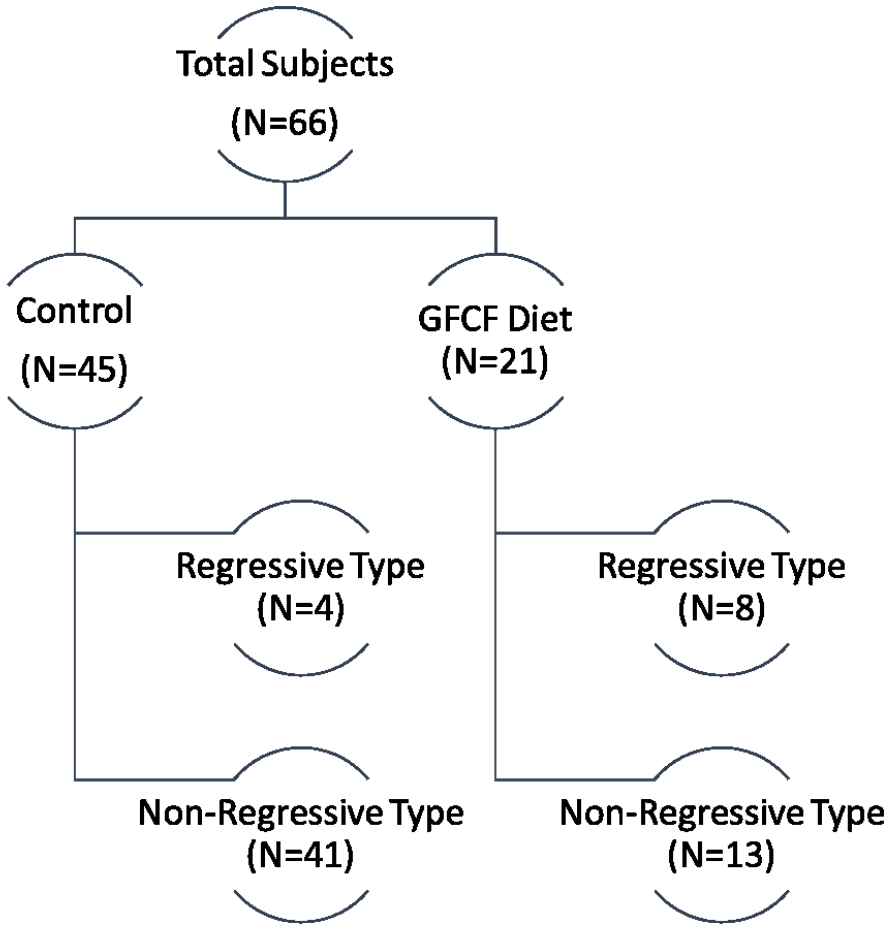

Figure 2. Breakdown of subject population into subtype of autism spectrum disorder. (GFCF = Gluten-free / Casein-free diet).

implementation of the GFCF diet[31-35]. While improvement of behaviors was reported in the GFCF group, no improvements were consistently noted in the control group. If anything, worsening or fluctuating behaviors were reported in the control group. While a concern for constipation and worsening gastrointestinal symptoms has been offered as a reason not to start a GFCF diet [39, 40], the data in this study suggests that a GFCF diet may actually improve the severity of gastrointestinal symptoms. Furthermore, no serious side effects were reported.

The exact mechanism as to why a GFCF diet may improve the symptoms in children with an ASD remains to be understood. One hypothesized mechanism of the GFCF diet's utility is the elimination of deleterious antibodies through the removal of ingested antigens (gluten and casein). In this cohort, though, none of the children were positive for the presence of the tissue transglutaminase antibody (which is used as a screening tool for celiac disease) or other celiac antibodies. In fact, none of the children could be defined, at least in the conventional sense, as having celiac disease. There is evidence that antibody seropositivity can both screen for and actually diagnose celiac disease in symptomatic children. While quantification of serum antibodies has long been used as a screening tool for celiac disease and endoscopy with biopsy has been used as the diagnostic gold standard, elevated "celiac" antibody titers such as tissue transglutaminase antibody may actually be sufficient to diagnose celiac disease in children[49,50]. In fact, duodenal histology consistent with celiac disease has been demonstrated to be correlated with seropositivity for tissue transglutaminase and endomysial antibody levels in children[51]. As such, by excluding children with seropositivity to tissue transglutaminase and endomysial antibody, all children with classic celiac disease were likely excluded from the study.

At the same time, the frequency of patients with sensitivity to gluten in the absence of classic "celiac" antibodies or histological findings seems to far outnumber those with seropositivity for antibodies or histological findings consistent with celiac disease[52]. To our knowledge, no studies have firmly established an explanation for this phenomenon. It is not clear whether children with ASDs still have a form of celiac disease or gluten-sensitivity that is not diagnosable with current techniques. The increased prevalence of antibodies to gliadin and casein in children with ASDs [26-29] provides one rational explanation as to why a diet devoid of gliadin and casein may lead to clinical improvement. Even in children without obvious presence of these antibodies, there may be an overall increased presence of auto-antibodies[53-57]. A gluten-free diet may reduce inflammation by eliminating antigens and may even be protective against auto-immunity[58, 59]. Although the sample size was too small to warrant statistical analysis, children with regressive ASDs seemed to respond disproportionately better to the GFCF diet. It may be that regressive-type ASDs, which are marked by pro-inflammatory immune dysfunction especially in the gut[60, 61], are sensitive to the anti-inflammatory effects of the GFCF diet. In addition, we speculate that regressive-type ASDs have a distinctly abnormal gut microbiota[62, 63] which may also be responsive to dietary interventions.

Obvious limitations of this study include its retrospective nature, lack of randomization, lack of a mechanism to ensure adherence to the GFCF diet, and not using a formal assessment tool for behavioral and language symptoms, and other interventions such as speech or behavioral therapies were not standardized among the two groups. The small number of children with regression-type ASDs also prohibited subgroup analysis for this variable. Still, based upon the data in this study, it appears that in a subset of children with an ASD and gastrointestinal symptoms the GFCF is a relatively well-tolerated intervention that may improve gastrointestinal symptoms and perhaps behaviors. This effect may be greater in children with a regressive ASD, but further research with a larger population would be needed to validate this hypothesis.

Acknowledgements
We thank Dr. Iona Monteiro (Division of Gastroenterology, Department of Pediatrics, Rutgers-New Jersey Medical School, Newark, NJ) for assisting in obtaining patients for this study. We thank Malky Korenblit for assistance in extracting data from patient charts.

Author Contributions: JK participated in study design, data collection, analysis, data interpretation, and wrote the initial draft of the manuscript. DH and XM participated in study design, data collection, analysis, data interpretation, and manuscript revision. KP participated in data analysis and preparation of tables and results section. All authors participated in preparing and approving the final version of the manuscript.

Authors' Note: The study was performed at Rutgers-New Jersey Medical School in Newark, New Jersey.

Declaration of Conflicting Interests: The authors have no conflicts of interest.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval: This study was approved by the Rutgers-New Jersey Medical School Investigational Review Board.

1. Marcus RN, Owen R, Kamen L, et al. A placebo-controlled, fixed-dose study of aripiprazole in children and adolescents with irritability associated with autistic disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2009;48(11): 1110-9.
2. Marcus RN, Owen R, Manos G, et al. Safety and tolerability of aripiprazole for irritability in pediatric patients with autistic disorder: a 52-week, open-label, multicenter study. *The Journal of clinical psychiatry*. 2011;72(9): 1270-6.
3. Shea S, Turgay A, Carroll A, et al. Risperidone in the treatment of disruptive behavioral symptoms in children with autistic and other pervasive developmental disorders. *Pediatrics*. 2004;114(5): e634-41.
4. McCracken JT, McGough J, Shah B, et al. Risperidone in children with autism and serious behavioral problems. *The New England journal of medicine*. 2002;347(5): 314-21.
5. Molloy CA and Manning-Courtney P. Prevalence of chronic gastrointestinal symptoms in children with autism and autistic spectrum disorders. *Autism*. 2003;7(2): 165-71.
6. Ibrahim SH, Voigt RG, Katusic SK, Weaver AL and Barbaresi WJ. Incidence of gastrointestinal symptoms in children with autism: a population-based study. *Pediatrics*. 2009;124(2): 680-6.
7. Rossignol DA. Novel and emerging treatments for autism spectrum disorders: a systematic review. *Annals of clinical psychiatry : official journal of the American Academy of Clinical Psychiatrists*. 2009;21(4): 213-36.
8. Smith RA, Farnworth H, Wright B and Allgar V. Are there more bowel symptoms in children with autism compared to normal children and children with other developmental and neurological disorders?: A case control study. *Autism*. 2009;13(4): 343-55.
9. Pang KH and Croaker GD. Constipation in children with autism and autistic spectrum disorder. *Pediatric surgery international*. 2011;27(4): 353-8.
10. Kushak RI, Lauwers GY, Winter HS and Buie TM. Intestinal disaccharidase activity in patients with autism: effect of age, gender, and intestinal inflammation. *Autism*. 2011;15(3): 285-94.
11. Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG and Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. *Autism research : official journal of the International Society for Autism Research*. 2012;5(2): 101-8.
12. McElhanon BO, McCracken C, Karpen S and Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics*. 2014;133(5): 872-83.
13. Chaidez V, Hansen RL and Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. *J Autism Dev Disord*. 2014;44(5): 1117-27.
14. de Magistris L, Familiari V, Pascotto A, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *Journal of pediatric gastroenterology and nutrition*. 2010;51(4): 418-24.
15. Finegold SM, Molitoris D, Song Y, et al. Gastrointestinal microflora studies in late-onset autism. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2002;35(Suppl 1): S6-S16.
16. Parracho HM, Bingham MO, Gibson GR and McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Journal of medical microbiology*. 2005;54(Pt 10): 987-91.
17. Finegold SM, Dowd SE, Gontcharova V, et al. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe*. 2010;16(4): 444-53.
18. Adams JB, Johansen LJ, Powell LD, Quig D and Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. *BMC gastroenterology*. 2011;11: 22.
19. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT and Conlon MA. Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. *Applied and environmental microbiology*. 2011;77(18): 6718-21.
20. Critchfield JW, van Hemert S, Ash M, Mulder L and Ashwood P. The potential role of probiotics in the management of childhood autism spectrum disorders. *Gastroenterology research and practice*. 2011;2011: 161358.
21. Ming X, Stein TP, Barnes V, Rhodes N and Guo L. Metabolic perturbance in autism spectrum disorders: a metabolomics study. *Journal of proteome research*. 2012;11(12): 5856-62.
22. Kang DW, Park JG, Ilhan ZE, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PloS one*. 2013;8(7): e68322.
23. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*. 2013;155(7): 1451-63.
24. Heberling CA, Dhurjati PS and Sasser M. Hypothesis for a systems connectivity model of Autism Spectrum Disorder pathogenesis: links to gut bacteria, oxidative stress, and intestinal permeability. *Medical hypotheses*. 2013;80(3): 264-70.
25. Hanson E, Kalish, L.A., Bunce, E., Curtis, C., McDaniel, S., Ware, J., Petry, J. Use of complementary and alternative medicine among children diagnosed with autism spectrum disorder. *J Autism Dev Disord*. 2007;37(4): 628.

26. Lau NM, Green PH, Taylor AK, et al. Markers of Celiac Disease and Gluten Sensitivity in Children with Autism. *PloS one*. 2013;8(6): e66155.
27. Kawashti MI, Amin OR and Rowehy NG. Possible immunological disorders in autism: concomitant autoimmunity and immune tolerance. *The Egyptian journal of immunology / Egyptian Association of Immunologists*. 2006;13(1): 99-104.
28. Reichelt KL, Ekrem, J., Scott, H. Gluten, milk proteins and autism: dietary intervention effects on behavior and peptide secretion. *Journal of Applied Nutrition*. 1990;42(1): 12.
29. Vojdani A, O'Bryan T, Green JA, et al. Immune response to dietary proteins, gliadin and cerebellar peptides in children with autism. *Nutritional neuroscience*. 2004;7(3): 151-61.
30. Elder JH. The gluten-free, casein-free diet in autism: an overview with clinical implications. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition*. 2008;23(6): 583-8.
31. Whiteley P, Rodgers J, Savery D and Shattock P. A gluten-free diet as an intervention for autism and associated spectrum disorders: preliminary findings. *Autism*. 1999;3(1): 45-65.
32. Harris C and Card B. A pilot study to evaluate nutritional influences on gastrointestinal symptoms and behavior patterns in children with Autism Spectrum Disorder. *Complementary therapies in medicine*. 2012;20(6): 437-40.
33. Pennesi CM and Klein LC. Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum disorder: based on parental report. *Nutritional neuroscience*. 2012;15(2): 85-91.
34. Knivsberg AM, Reichelt KL, Hoien T and Nodland M. A randomised, controlled study of dietary intervention in autistic syndromes. *Nutritional neuroscience*. 2002;5(4): 251-61.
35. Whiteley P, Haracopos D, Knivsberg AM, et al. The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. *Nutritional neuroscience*. 2010;13(2): 87-100.
36. Johnson CR, Handen BL, Zimmer M, Sacco K and Turner K. Effects of gluten free/casein free diet in young children with autism: A pilot study. *Journal of Developmental and Physical Disabilities*. 2011;23(3): 213-25.
37. Elder JH, Shankar M, Shuster J, Theriaque D, Burns S and Sherrill L. The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial. *Journal of autism and developmental disorders*. 2006;36(3): 413-20.
38. Millward C, Ferriter M, Calver S and Connell-Jones G. Gluten- and casein-free diets for autistic spectrum disorder. *The Cochrane database of systematic reviews*. 2008;(2): CD003498.
39. Mulloy A, Lang R, O'Reilly M, Sigafoos J, Lancioni G and Rispoli M. Gluten-free and casein-free diets in the treatment of autism spectrum disorders: a systematic review. *Research in Autism Spectrum Disorders*. 2010;4(3): 328-39.
40. Marí-Bauset S, Zazpe I, Mari-Sanchis A, Llopis-González A and Morales-Suárez-Varela M. Evidence of the Gluten-Free and Casein-Free Diet in Autism Spectrum Disorders A Systematic Review. *Journal of child neurology*. 2014: 0883073814531330.
41. Mulloy A, Lang R, O'Reilly M, Sigafoos J, Lancioni G and Rispoli M. Addendum to "gluten-free and casein-free diets in treatment of autism spectrum disorders: A systematic review". *Research in Autism Spectrum Disorders*. 2011;5(1): 86-8.
42. Hu VW, Addington A and Hyman A. Novel autism subtype-dependent genetic variants are revealed by quantitative trait and subphenotype association analyses of published GWAS data. *PloS one*. 2011;6(4): e19067.
43. Volkmar FR, State M and Klin A. Autism and autism spectrum disorders: diagnostic issues for the coming decade. *Journal of Child Psychology and Psychiatry*. 2009;50(1-2): 108-15.
44. Tager-Flusberg H and Joseph RM. Identifying neurocognitive phenotypes in autism. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*. 2003;358(1430): 303-14.
45. Buie T, Campbell DB, Fuchs GJ, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics*. 2010;125(Supplement 1): S1-S18.
46. Association AP. Diagnostic and statistical manual of mental disorders DSM-IV-TR fourth edition (text revision). 2000.
47. Schneider CK, Melmed RD, Barstow LE, Enriquez FJ, Ranger-Moore J and Ostrem JA. Oral human immunoglobulin for children with autism and gastrointestinal dysfunction: a prospective, open-label study. *Journal of autism and developmental disorders*. 2006;36(8): 1053-64.
48. Developmental DMNSY and Investigators P. Prevalence of autism spectrum disorder among children aged 8 years-autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *Morbidity and mortality weekly report Surveillance summaries (Washington, DC: 2002)*. 2014;63(2): 1.
49. Barker CC, Mitton C, Jevon G and Mock T. Can tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations? *Pediatrics*. 2005;115(5): 1341-6.
50. Klapp G, Masip E, Bolonio M, et al. Celiac disease: the new proposed ESPGHAN diagnostic criteria do work well in a selected population. *Journal of pediatric gastroenterology and nutrition*. 2013;56(3): 251-6.
51. Donaldson MR, Firth SD, Wimpee H, et al. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2007;5(5): 567-73.
52. Lundin KE and Alaedini A. Non-celiac gluten sensitivity. *Gastrointestinal endoscopy clinics of North America*. 2012;22(4): 723-34.
53. Frye R, Sequeira J, Quadros E, James S and Rossignol D. Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular psychiatry*. 2013;18(3): 369-81.
54. Singh VK and Rivas WH. Prevalence of serum antibodies to caudate nucleus in autistic children. *Neuroscience letters*. 2004;355(1): 53-6.
55. Vojdani A, Campbell A, Anyanwu E, Kashanian A, Bock K and Vojdani E. Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, Chlamydia pneumoniae and Streptococcus group A. *Journal of neuroimmunology*. 2002;129(1): 168-77.
56. Mostafa GA and Al-Ayadhi LY. The relationship between the increased frequency of serum antineuronal antibodies and the severity of autism in children. *european journal of paediatric neurology*. 2012;16(5): 464-8.
57. Singh VK, Warren R, Averett R and Ghaziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. *Pediatric neurology*. 1997;17(1): 88-90.
58. Cosnes J, Cellier C, Viola S, et al. Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet. *Clinical Gastroenterology and Hepatology*. 2008;6(7): 753-8.
59. Soares FLP, de Oliveira Matoso R, Teixeira LG, et al. Gluten-free diet reduces adiposity, inflammation and insulin resistance associated with the induction of PPAR-alpha and PPAR-gamma expression. *The Journal of nutritional biochemistry*. 2013;24(6): 1105-11.
60. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I and Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, behavior, and immunity*. 2011;25(1): 40-5.
61. Torrente F, Anthony A, Heuschkel RB, Thomson MA, Ashwood P and Murch SH. Focal-enhanced gastritis in regressive autism with features distinct from Crohn's and Helicobacter pylori gastritis. *The American journal of gastroenterology*. 2004;99(4): 598-605.
62. Finegold SM, Downes J and Summanen PH. Microbiology of regressive autism. *Anaerobe*. 2012;18(2): 260-2.
63. Finegold SM. Desulfovibrio species are potentially important in regressive autism. *Medical hypotheses*. 2011;77(2): 270-4.