

Objective determination of markers of gluten and casein sensitivity among children with autistic spectrum disorders (ASD)

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Background:

The Biomedical model of autism management, pioneered by the founders of the Autism Research Institute, highlights a gluten free/casein free diet as an important first step in the process of treating the disorder. The Autism Research Institute shows through its Parent Ratings of Biomedical Interventions that 66% of 2,561 children who tried the diet found it beneficial in producing (behavioural) changes. (Autism Research Institute 2008). Opioid by-products resulting from incomplete digestion of gluten and/or casein (gliadorphins and casomorphins, respectively) have been proposed as a mechanistic basis by which gluten and casein impact ASD. Likewise, immunological reactivity to gluten and casein (assessed through determination of plasma IgG levels to these proteins) has been implicated as a contributing factor to clinical responsiveness from their elimination in the diet.

Purpose:

This study sought to determine the prevalence of gluten and casein sensitivity (assessed using the urinary organic peptides test and determination of plasma IgG levels) among children with ASD.

Methods:

This study reports on a retrospective collection of test results from 26 randomly selected ASD children tested at Touchstone Naturopathic Centre from November 2007 to August 2008. The children range in age from two to 13 years; 25 males and one female were studied. Inclusion criteria were presentation to the Touchtone Naturopathic Centre with an ASD diagnosis, and completion of one or both of the objective tests comprising the principle endpoint measure of the study; urinary peptides and IgG antibody food allergy testing; both performed by the Great Plains Laboratory in Lenexa, KA. Thirteen tests were done for the two principle urinary peptides, casomorphin and gliadorphin. Eighteen IgG antibody food allergy tests reporting on reactions to 96 foods were performed; only results for milk, cheese, casein, wheat, oats, gluten and gliadin are reported here. Of the 19 children retained in the study group, two were following the SCD or Specific Carbohydrate Diet and eight were in the early phases of starting the GF/CF diet. Most children were also taking a multivitamin supplement, probiotic supplement and fish- derived Omega-3 oil supplement at this time.

Table 1: Results of urinary peptide testing

Urinary Peptide	Normal range*	Number of patients tested	Number (%) of patients positive	Number (%) of patients with range* 0.57-1.0	Number (%) of patients with range* 1.0-1.5	Number (%) of patients with range* 1.5-2.0	Number (%) of patients with range* >2.0
Casomorphin	<0.56	13	6 (46)	2 (15)	1 (8)	2 (15)	1 (8)
Gliadorphin	<0.58	13	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*(ng/ml urinary peptide) / (mg/dl creatinine)

Table 2: Results of IgG antibody food allergy testing

IgG antibody tested	Number of patients tested	Number (%) of patients positive
Milk	18	14 (78)
Cheese	18	10 (56)
Casein	18	11 (61)
Wheat	18	15 (83)
Oats	18	11 (61)
Gluten	18	14 (78)
Gliadin	18	10 (56)

Table 1: Results of urinary peptide testing

Magnitude of impact from GF/CF diet	None	Mild	Moderate	Good
Number (%) of patients	2 (10)	6 (32)	7 (37)	4 (21)

Results:

Quantifiable levels of casomorphin were detected in the urine of six (46%) of the 13 patients tested. No patient tested positive for the presence of gliadorphin in the urine (see Table 1).

Eighteen patients underwent testing for plasma IgG antibodies to a battery of 96 common foods and selected food proteins. Results for IgG antibodies to milk, cheese, casein, wheat, oats, gluten and gliadin are reported in Table 2. All 18 children tested positive for IgG antibodies to at least one

of the listed foods. Four children (22%) tested positive for two foods. Five children (28%) tested positive for three foods. Five children (28%) tested positive for four foods, and three children (17%) tested positive for five foods.

Overall clinical impression of GF/CF diets in children with ASD is quite impressive. A significant subset of children were observed to respond favourably to dietary elimination of gluten and casein containing foods. We present a subjective ranking of responses of patients, based on parent reporting and clinical observation of impact from the GF/ CF diet

intervention (see Table 3). Outcomes, as reported by parents and observed clinically, included the following:

- Increased speech and/or language use: Increased effort to speak or communicate (including pointing for desired things such as food or toys), increased use of multiple words/sentences, proper use of nouns (e.g. Mother), increased two-way communication.
- Improved social behaviour: Patients demonstrated improved interaction with siblings and other children in daycare, home and school settings. These improvements included better tolerance for group sessions (e.g. Circle Time) and initiating play with other children.
- Reduced gastrointestinal symptoms: Better consistency of stools, less odour, reduced bloating.
- Improved Sleep: Both in terms of onset of sleep and maintaining sleep through the night
- Reduced stimming or repetitive behaviours.

Discussion:

Opioid peptide byproducts of gluten and casein, as well as immune system production of antibodies, specifically IgG and occasionally IgA and IgE, are discussed as the mechanistic basis for deleterious impact of gluten and casein containing foods among individuals with ASD. The theory of opioid-peptide compounds impacting neurologic/psychiatric disorder was first proposed by Dohan and colleagues in 1976. This team observed elevated rates of celiac disease among patients with schizophrenia (Dohan 1976). A team of psychiatrists from John Hopkins School of Medicine recently presented a review of gluten and schizophrenia (Kalaydjian 2006). Observational evidence is presented correlating surpluses of grain in the food supply with increased hospital admissions for schizophrenia, and conversely reductions in hospital admissions for schizophrenia during times of grain shortages. The team also presents data highlighting immense clinical benefit from gluten elimination among patients with schizophrenia, but that this benefit is confined to a modest subset of patients.

In the late 1980's and early 1990's, several European-based research teams reported on the presence of gliadorphin and casomorphin in urine of individuals with neuropsychiatric disorders (Knivsberg 1990, Reichelt 1990, Reichelt 1991, Reichelt 1994, Shattock 1990). Based on clinical outcomes in Europe and North America, the opioid-peptide theory became the primary explanation for the benefits from GF/CF diets in ASD children. This theory gained momentum on the strength of North American-based research by Robert Cade of the University of Florida, associating a selection of urinary peptides with behavioural changes in animal models (Sun 1999a) and humans (Sun 1999b). Elevated levels of opioid-peptides such as casomorphin or gliadorphin may occur because of an enzyme deficiency, specifically DPP IV (Dipeptidyl peptidase IV) (Elgun 1999, Smith 1990). Further support for the theory of deleterious impact from opioid peptides in autism was provided

by a clinical trial which demonstrated efficacy from low dosages of naltrexone (an anti-addiction medication) in autistic children (Bovard 1995).

Based on the results of the present study, the opioid peptides casomorphin and gliadorphin appear to be minor contributors to the mechanistic basis for benefit from dietary elimination of gluten and casein. Forty six percent of patients tested positive for urinary gliadorphin, but only half of these (three of 13 patients or 23%) were found to possess a concentration likely to explain positive clinical outcome from dietary elimination of casein. Not a single patient tested positive for gliadorphin. We propose that a modest subset of patients is likely to derive benefit from reduction in exposure to gliadorphins and casomorphins.

In recent years, immune complexes or antibodies derived from different foods have been investigated for their impact in a variety of health complaints. Intact "cow's milk proteins" have been clearly established as factors impacting the prevalence of asthma, wheeze and eczema in newborn infants, as reviewed by the Cochrane library of systematic reviews (Ram 2002). The American Academy of Pediatrics has recommended hydrolyzed cow's milk formula for all infants at high risk of atopy since 2000 (American Academy of Pediatrics 2000). A team of pediatric gastroenterologists from Italy claims a 70% success rate in treating pediatric constipation through elimination of dairy products from the diet (Carroccio 2006).

Immunological reactivity to gluten has been implicated in a wide array of autoimmune disorders as well as neuropsychiatric disorders. Kull and colleagues demonstrated 34% of patients with a diagnosis of ulcerative colitis to be positive for IgG antigliadin antibodies, relative to 16% of patients with a diagnosis of IBS and 0% of otherwise healthy controls (Kull 1999). Berti (2000) demonstrated 0.25% of random blood donors, 0.75% of patients with non gastrointestinal malignancies, and 3.4% of patients with autoimmune thyroiditis to be positive for celiac disease (positive for IgA class antiendomysium antibodies). Wahnschaffe (2007) found 98% of patients with celiac disease not following a gluten free diet, 55% of patients with celiac disease following a gluten free diet, 35% of patients with a diagnosis of IBS, and 18% of patients with a diagnosis of inflammatory bowel disease to be positive for antigliadin IgG antibodies.

Elimination of dietary gluten has demonstrated impressive clinical efficacy as a management strategy for a number of autoimmune disorders, sometimes directly correlated to the presence of antigliadin IgG antibodies (Wahnschaffe 2007), otherwise simply imposed as an intervention to observe clinical outcome without objective evaluation of immune reactivity (Gaby 1998, Hafstrom 2001).

It is therefore probable that immunological reactivity to casein and/or gluten is a significant contributor to the mechanistic basis for observed improvements upon their elimination in neuropsychiatric disorders. As previously discussed, gluten has been directly implicated in the pathogenesis of schizophrenia (Dohan 1976, Kalaydjian 2006). A team of neurologists from England has published a series of papers providing impressive evidence for links between gluten and ataxia, leading the team to coin the term "gluten ataxia" to describe a significant subset of patients afflicted with this progressive, debilitating disorder (Hadjivassiliou 2006a, Hadjivassiliou 2006b, Sanders 2003, Wilkinson 2005).

Specific to autism, Cade (2000) found gliadin and casein antibodies to be present in 86% and 90% of autistic children studied, respectively. The team reported significant clinical

improvement in 81% of patients during the three months of treatment with a GF/CF diet period. A more recent study found elevated levels of antibodies to gluten and casein in a population of 35 autistic children relative to 21 of the subjects neurotypical siblings (Trajkovski 2008). Kawashti (2006) performed IgG antibody testing for gluten and casein in 30 children with Autism. 83% were observed to be positive for casein, while 50% were positive for gluten. The results of the present study closely parallel these earlier reports (See Table 2).

Therefore, it appears the presence of plasma IgG antibodies to gluten/casein, and foods containing them, are more important markers for guiding the clinical application of gluten and casein free diets relative to the presence of gliadorphin and casomorphin in the urine. ■

References

- American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics*. 2000;106(2 Pt 1):346-9.
- Autism Research Institute. Parent ratings of behavioral effects of biomedical interventions. www.autism.com Accessed Oct 2008.
- Berti I, Trevisiol C, Tommasini A, Città A, Neri E, Geatti O, Giammarini A, Ventura A, Not T. Usefulness of screening program for celiac disease in autoimmune thyroiditis. *Dig Dis Sci*. 2000;45(2):403-6.
- Bovard, et al. Low-dose Naltrexone effects on plasma chemistries and clinical symptoms in autism: a double blind, placebo-controlled study. *Psychiatry Research* 1995; 58: 191-200.
- Bushara, KO. Neurologic presentation of celiac disease. *Gastroenterology*. 2005 Apr; 128 (4 Suppl 1):592-7.
- Cade, R. Autism and Schizophrenia: intestinal disorders. *Nutritional Neuroscience* 3: 57-72, 2000.
- Carroccio A, Iacono G. Review article: Chronic constipation and food hypersensitivity – an intriguing relationship. *Aliment Pharmacol Ther*. 2006;24(9):1295-304.
- Dohan, FC. The possible pathogenic effect of cereal grains in schizophrenia – Celiac disease as a model. *Acta Neurol*. 31:195, 1976.
- Elgun, S et al. Dipeptidyl peptidase IV and adenosine deaminase activity. Decrease in depression. *Psychoneuroendocrinology* 24(8): 823-32, 1999 Nov.
- Gaby AR. The role of hidden food allergy/intolerance in chronic disease. *Altern Med Rev*. 1998;3(2):90-100.
- Hadjivassiliou M, Kandler RH, Chattopadhyay AK, Davies-Jones AG, Jarratt JA, Sanders DS, Sharrack B, Grünewald RA. Dietary treatment of gluten neuropathy. *Muscle Nerve*. 2006 (a);34(6):762-6.
- Hadjivassiliou M, Grünewald RA, Kandler RH, Chattopadhyay AK, Jarratt JA, Sanders DS, Sharrack B, Wharton SB, Davies-Jones GA. Neuropathy associated with gluten sensitivity. *J Neurol Neurosurg Psychiatry*. 2006(b);77(11):1262-6.
- Hafström I, Ringertz B, Spångberg A, von Zweigbergk L, Brannemark S, Nylander I, Rönnelid J, Laasonen L, Klareskog L. A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: the effects on arthritis correlate with a reduction in antibodies to food antigens. *Rheumatology (Oxford)*. 2001;40(10):1175-9.
- Kalaydjian AE, Eaton W, Cascella N, Fasano A. The gluten connection: the association between schizophrenia and celiac disease. *Acta Psychiatr Scand*. 2006;113(2):82-90.
- Kawashti MI, Amin OR, Rowehy, NG. Possible Immunological disorders in autism: concomitant autoimmunity and immune tolerance. *Egypt J Immunol*. 2006; 13(1):99-104.
- Knivsberg, A et al. Dietary Intervention in Autistic Syndromes. *Brain Dysfunction* 3: 315-327, 1990.
- Kull K, Uibo O, Salupere R, Metsküla K, Uibo R. High frequency of antigliadin antibodies and absence of antireticulin and antiendomysium antibodies in patients with ulcerative colitis. *J Gastroenterol*. 1999;34(1):61-5.
- Ram FS, Ducharme FM, Scarlett J. Cow's milk protein avoidance and development of childhood wheeze in children with a family history of atopy. *Cochrane Database Syst Rev*. 2002;(3):CD003795.
- Reichelt, K et al. Gluten, milk proteins and autism: dietary interventions effects on behavior and peptide secretion. *J App. Nutr*. 42: 1-11, 1990.
- Reichelt, K et al. Probable etiology and possible treatment of childhood autism. *Brain Dysfunction* 4: 308-319, 1991.
- Reichelt, K et al. Nature and consequences of hyperpeptiduria and bovine casomorphins found in autistic syndromes. *Developmental Brain Dysfunction* 7: 71-85, 1994.
- Rumbo, M et al. Evaluation of celiac disease serological markers in Down syndrome patients. *Dig Liver Dis*. 2002 Feb; 34(2): 116-121.
- Sanders DS, Patel D, Stephenson TJ, Ward AM, McCloskey EV, Hadjivassiliou M, Lobo AJ. A primary care cross-sectional study of undiagnosed adult coeliac disease. *Eur J Gastroenterol Hepatol*. 2003;15(4):407-13.
- Shattock P et al. Role of neuropeptides in autism and their relationships with classical neurotransmitters. *Brain Dysfunction* 3: 328-345, 1990.
- Smith MW, Phillips AD. Abnormal expression of dipeptidyl peptidase IV activity in enterocyte brush-border membranes of children suffering from coeliac disease. *Exp Physiol* 1990 Jul; 75(4): 613-6.
- Sun, Z, Cade, R. A peptide found in schizophrenia causes behavioral changes in rats. *Autism* 3: 85-96, 1999a.
- Sun Z, Cade, R et al. Beta-casomorphin induces Fos-like reactivity in discrete brain regions relevant to schizophrenia and autism. *Autism* 3: 67-84, 1999b.
- Trajkovski, V et al. Higher Plasma Concentration of Food-Specific Antibodies in Persons With Autistic Disorder in Comparison to Their Siblings. *Focus on Autism and Other Developmental Disabilities*, Vol. 23, No. 3:176-185, 2008.
- Vojdani, A, Pangborn, JG et al. Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major instigators of autoimmunity in autism. *Int J Immunopathol Pharmacol*. 2003 Sep-Dec; 16(3):189-99.
- Wahnschaffe U, Schulzke JD, Zeitl M, Ullrich R. Predictors of clinical response to gluten-free diet in patients diagnosed with diarrhea predominant irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2007;5(7):844-50.
- Wilkinson ID, Hadjivassiliou M, Dickson JM, Wallis L, Grünewald RA, Coley SC, Widjaja E, Griffiths PD. Cerebellar abnormalities on proton MR spectroscopy in gluten ataxia. *J Neurol Neurosurg Psychiatry*. 2005;76(7):1011-3.