Introduction and Rationale

We previously reported that, when compared to controls, a significant number of autistic family members have lower than normal serum levels of alpha-1 antitrypsin (AAT) (1). We have also reported that a significant number of autistic children with GI disease have anti neutrophil cytoplasmic antibodies (ANCA) (both anti-PR3 and anti-MPO), and that there is a relationship between individuals with ANCA and severity of intestinal disease (2). Recently, we reported preliminary data suggesting that a significant number of autistic children with chronic digestive disease have low serum AAT, anti-PR3 ANCA and high serum PR3, which correlate with high severity of GI disease when compared to controls (3). This suggests that low AAT levels may result in high levels of PR3, which may, in turn be associated with the formation of ANCA.

There is evidence to suggest that low levels of AAT (AAT deficiency) may be associated with the etiology of Celiac Disease (CD) (4-11), and conflicting evidence to support the relationship between CD and autism (17,18).

The incidence of CD in newborns may be as high as 1 in 100 births in the U.S. (12-15), depending on the study population. However, the incidence in children with other autoimmune disorders may be as high as 1 in 12 (16). It would also be expected that a higher incidence of CD would be found in children with existing GI disease.

This study will attempt to determine if there is a relationship between CD and autism in autistic children with GI disease, and, if so, determine if the presence of CD biomarkers correlates with low levels of AAT and/or ANCA(s).

Experimental Design

There is evidence to suggest that anti-transglutaminase screening (using the IgA+/IgG isotype) is the most sensitive and specific serological assessment of CD (10,11).

In order to determine if there is a relationship between CD and autism, we will test 200 autistic children with GI disease and 200 age and gender matched children without GI disease (100 autistic, 100 non autistic) for anti-human transglutaminase IgG and IgA using ELISAs (Kits from Immco Diagnostics, Buffalo, NY).

In order to compare this data with serum AAT concentrations and ANCA,
will test the same 200 autistic children with GI disease and 200 children without GI disease (100 autistic, 100 non autistic) for serum AAT and ANCA (both PR3 and MPO) using ELISAs (AAT Kits from ALPCO Immunodiagnostics, Salem, NH and ANCA Kits from Immco Diagnostics, Buffalo, NY).

In order to assess the relationship between CD, AAT, ANCA and autistic children with GI disease, we will then compare CD autoantibody, AAT and ANCA to GI disease severity (using endoscopic assessment – Thoughtful House) and diet (Thoughtful House assessment) of the autistic children with GI disease.

Participants and Location

Serum samples from autistic children with GI disease, as well as endoscopic and dietary assessment will be obtained from the Thoughtful House, Austin, Texas, and control samples will be purchased from Autistic Genetic Resource Exchange (AGRE).

Budget

Estimated Cost:

ELISA kits and AGRE Serum – Total = $8000.00

1. Anti-human transglutaminase IgG and IgA ELISA Kits (Immco Diagnostics, Buffalo, NY). Each kit can measure approximately 100 samples. 8 kits *
   $250.00/kit = $2000.00
2. AAT Kits. Each kit can measure approximately 100 samples. . 8 kits *
   $250.00/kit = $2000.00
3. ANCA Kits. . Each kit can measure approximately 100 samples. . 8 kits *
   $250.00/kit = $2000.00
4. AGRE serum. 200 control serums = $2000.00

Discussion

A recent study at Tehran University of Medical Sciences suggested that food allergies, often associated with autism, had no connection to the gluten intolerance experienced by people with celiac disease (17). The problems associated with this study include small sample size and questionable marker identification for CD. Only 34 autistic children and controls were screened for CD. With an expected CD incidence of 1 in 100, this number is too low (especially because this study includes a general population of autistic children), and the group measured anti-gliadin antibodies (these may not be the best markers for CD). Even with that said, the researchers found 4 autistic children and two controls with anti-gliadin antibodies. If this study were expanded to include many more individuals, the scientists may have found a significant difference in the biomarker between the
experimental and control groups. Our study includes a higher number of children from a selected group (with GI disease), so, if there is a relationship between CD and autism (particularly in autistic children with GI disease), we have an opportunity to identify a significant number of individuals with autoantibodies. In fact, in contrast to the study above, Barcia et al, at The University of Brussels in 2008 (18), retrospectively evaluated 150 autistic subjects (123 males, 27 females; mean age 6 years 8 months). Five out of one hundred and fifty subjects (3.3%) were diagnosed with CD, which was significantly higher ($p = .014$) than CD prevalence for the general pediatric population of 1:106 (Binomial Test). They concluded that all children with autism should be screened for CD, even if no gastrointestinal symptoms are present and that more studies are necessary to clarify autism and CD association, CD being a very frequent condition in the general population.

This preliminary study will attempt to determine whether CD is associated with autism, particularly autistic children with GI disease. It will also attempt to demonstrate whether AAT deficiency is associated with celiac disease, ANCA autoimmunity and/or autism. If this association does exist, could the subgroup of children with low serum AAT be more susceptible to a high gluten or casein diet? Could AAT therapy have an affect on dietary susceptibility? These are questions, which could be answered in future studies.

References

1. Russo, A.J., Neville, L, Wroge, C, Low Serum Alpha-1 Antitrypsin (AAT) in Family Members of Individuals With Autism Correlates with PiMZ Genotype, Biomarkers, accepted for publication, September 19, 2008.
8. Thomas DW, Sinatra FR, Merritt RJ Random fecal alpha-1-antitrypsin


