Beneficial Effects of Enzyme-based Therapy for Autism Spectrum Disorders

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Abstract
Autism is a developmental disease usually manifesting within the first three years of life. To date, no causative agent has been found. Similarly, treatment options have been limited. Of the treatment options available, a number of them have been nutritionally based in an attempt to address one or more of the theories regarding the etiology of the disease. A pilot study was undertaken to address the exorophin (exogenous opiates type peptides) theory of autism by treatment with digestive enzyme therapy. Patients were chosen for inclusion in the study based on a diagnosis placing them in the category of the autism spectrum disorders (ASD). The diets were supplemented with a novel dietary enzyme formulation, ENZYM-ED, for a period of twelve weeks. Progress was tracked according to the Symptom Outcome Survey (SOS) method of symptom charting and presented in a table for further analysis. Results: The novel enzyme formula, ENZYM-ED, beneficially and safely affected all 13 of the parameters measured. Improvements were noted up to 90%, depending on the parameter measured, of the respondents who completed the entire course of therapy. Statistical analysis revealed that even assuming an extremely high baseline, twelve of the thirteen parameters were significant improvements.

Conclusion: The novel enzyme formula ENZYM-ED was effective at improving autistic symptoms such as Socialization. These results indicate that further controlled studies are warranted.

Introduction
It has been estimated that 5:10,000 to 1:300 (though the primary author believes this number is much higher, especially in 'autism clusters') suffer from autism with an initial manifestation of symptoms by age three. While the exact cause of autism remains elusive, considerable advances have been made in recent years. These advances come from a study of the geographic localization, biological, and psychological aspects of the disease. From these studies, several theories have emerged.
negatively affect absorption of minerals such as zinc, calcium, magnesium, copper, manganese, and iron. Phytase supplementation results in greater bioavailability of these important minerals.

The only non-enzyme constituent of the blend is galactose. Galactose was included to function as a genochemical. Genochemicals are naturally occurring compounds, which are able to beneficially affect gene expression. The inclusion of galactose in the formulation is based on three different ideas. The first was the report by Smith et al. that galactose can increase the expression of dipeptidyl peptidase-IV (DPP IV or CD26) in murine enterocytes. The second is based on the finding that adding glucosamine could increase the level of transcription and translation of important genes. Both transcription and translation are important for a nutrient to be able to affect the levels of a protein made from the DNA. Wang's work demonstrates clearly, elegantly, and definitively, that the concept of genochemicals is sound. By adding glucosamine to the diet, not only did they get more RNA, but they got more protein as well. The corollary is that by adding galactose, more DPP IV can be made. This is the third work and is based on Brudnak's theory of genochemicals suggesting that because the addition of glucosamine to a diet can increase the expression of leptin (fat hormone) in the body, the addition of galactose may have a similar effect on DPP IV.

Genochemicals describe nutrients, which can affect the structure of a gene, how well the gene products (protein and sometimes RNA) works, and/or how much of it is made. That is to say, genochemicals cannot just replace substances which may be missing (e.g., an enzyme diminished by mutation), but actually alter the expression and functionality of gene products in, and resulting from, genomic multi-level nutrient-sensing pathways. The last category is where the inclusion of the galactose comes into play. In this case, the genochemical would be galactose.

Galactose appears to be able to increase the expression of the Dipeptidyl peptidase IV gene. This means adding galactose can increase the amount of DPP IV that is present. If, in autistics, the situation is not that there is absolutely zero DPP IV made, due to a mutated gene or regulatory element, but rather that the gene has been silenced or attenuated (down-regulated), then the addition of galactose has the potential to reverse or circumvent that. The caveat being that the gene needs to at least be functional and this is discussed below. Indeed, DPP IV is thought to be down regulated in autistics and is currently being used as a diagnostic marker for the disease. However, that is not the only place DPP IV, and DPP IV-like enzymes, are expressed and may prove to be secondary to other sources. A growing body of evidence indicates that certain probiotics in the human gastrointestinal tract, have an enormous amount of DPP IV-type activity. That is to say, the probiotics in the human gut can digest the same peptides as the products, which are currently supplied as being fungal in source.

The present study was undertaken to determine the effectiveness of the rationale above as gauged by 13 markers assessed in 22 patients. The results of the use of ENZYM-ED to treat autistics are presented below. Statistical analysis suggests that the enzyme formulation is both sound and effective for the treatment of autism spectrum disorders. Additionally, this study shows that enzymes can be formulated with a rationale, and studied first in pilot studies such as this one, prior to full-blown clinical studies.

Materials and Methods

Study Protocol and Human Subjects: The study protocol was approved by a Medical Review Board. The legal guardians of all participants signed appropriate "informed consent" forms. The study was carried out with the highest standards of ethics. Initially, 46 patients between the ages of 2 and 21 were chosen for the study. Of those that started the study, 22 remained for the entire twelve-week period. For data analysis, only those subjects who...

### Table 1. Percentage of Patients Showing Improvement

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Table 1. Percentage of Patients Showing Improvement. An observer scored 22 patients for the 13 parameters listed. The column "Start" is the total initial score as a percentage for improvement for each parameter. These were not actually scored and are assumed to be zero for each. Scores were taken every two weeks, totaled, and indicated as a percentage of the total. "Best case" P-value represents the significance based on a Student's T-test using the initial "Start" of zero. The "Worst case" P-value represents the significance based on a Student's T-test using the initial Week 1-2 reading as the base-line. The two groups analyzed for both Best-case and Worst-case were the initial Start vs. W 1-2, 3-4, 5-6, 7-8, 9-10, 11-12 percentages. P < 0.05 are considered significant.
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completed the entire course of therapy were included. All raw data is kept on file for public inspection at the Autism Research Institute (ARI), San Diego, CA.

Enzymes: All enzymes were formulated and manufactured by MAK Wood, Inc (Grafton, WI). Enzymes were copacked (encapsulated and bottled) to be free of milk, casein, wheat, gluten, gelatin, corn, soy, egg, yeast, sugar, starch, MSG, stevates, palmnates, artificial sweeteners, colors or flavors, preservatives, salicylates and other common allergens. The following enzymes and activities were included: Casein Glutensase 10,000 AU; Bromelain 230 BTU; Acid Fast Protease 100 SAPU; Lactase 330 LacU; Phytase 125 U; Galactose (as Genommeptic®) 100 mg.

Dosing: The enzymes were to be taken at the beginning of each meal (or early on into the meal). The capsules were swallowed whole or pulled apart and the contents mixed with the first few bites of food or in beverages. One half to one capsule with each meal was suggested with a gradual increase.

Assay: Parents, guardians, teachers, or therapists were asked to complete an SOS form every two weeks. They evaluated the thirteen different parameters of function and behavior in the child.

Statistical analysis: A Student's T-test for two groups was used for the statistical analysis. (A Java Implementation by Bryan Lewis, Kent State University, 1997)11 The significance is shown as the “P-value” for the two groups. The two groups were considered to be Before and After treatment with enzymes. The analysis was run in two ways. The first is the column designated “Best Case” in Table 1. This column represents a comparison of before and after treatment assuming that all patients scored an initial zero for the before treatment score. The second is shown in the column designated “Worst Case” in Table 1. This column represents a comparison of before and after treatment assuming that all patients scored an initial marking equal to the 1-2 week period score. This was done to elevate the base-line.

Results
Of the 46 patients that initially started the treatment, 17 dropped out during the first few weeks of the twelve-week trial. Those that remained for the entire twelve-weeks were included in the data analysis. For each of the thirteen parameters, patients were assayed by the following 0-4 scale: 0=none; 1=possible; 2=moderate; 3=significant; 4=great. The results in Table 1 list the percentage of patients that scored between a moderate and significant improvement by a guardian.

Patients who stopped the study in the first few weeks, reported a number of reasons including: need to start other therapies; family illness; personal issues; changed physicians; did not tolerate the taste of the supplement and/or no change in symptoms. Additionally, there were six patients who had “adverse responses” including hyperactivity/increased aggression, increased agitation, (one), diarrhea/loose stools (two), provocation of red ears and cheeks (one), increased hunger (one), and stopped eating (one). Approximately 40% (data not shown) of the participants were following a gluten-free and casein-free diet but all were instructed not to change their diet during the treatment.

An overwhelmingly positive trend is seen for each of the parameters. The two greatest improvements were seen in Socialization and Hyperactivity with 90% and 80% improvements respectively. The lowest improvements were for Stimming, Speech, and Sound Sensitivity, each scoring 50%.

Statistical analysis (Student's T-test) using zero for the base-line in the “Best case” column indicated that all scores were significant. The P-values ranged from 0.004429, for Sound Sensitivity, to 8.75 x 10^{-4} for Stimming. Because the zero point was an assumed number, a further round of analysis was pursued. Here, as indicated in column “Worst case” the base-line was chosen to be the same as that of the initial 1-2 week rating. All parameters except for one, Sound Sensitivity, were again shown to be statistically significant.

Discussion
There were a number of special conditions experienced with the study, and may have contributed to the dropout rate. Most of those who dropped out, did so early on in the study. The majority of those who dropped out had reasons other than negative effects of the study on the patient. Also, the families were instructed not to start any new therapies while in the study. This proved to be an unexpected problem as several families apparently had a strong desire to try other approaches. This was an interesting development and will need to be considered when designing follow-up studies. Despite the dropout rate, it is believed that the ENZYM-ED formulation was generally well-tolerated as only a few respondents reported adverse effects which may be directly attributed to the enzyme formulation.

While other studies using enzymes have been undertaken, a Medline search revealed that none have been reported in the literature at this time. While the present study is similar in structure, the novelty of the enzyme formulation is pronounced. In addition to adding several new enzymes, this is the first reporting of the therapeutic potential of genomceuticals.

The functioning of galactose is believed to be at two levels. The first is as a genommeptic. Here, it is believed to be increasing the gut expression of the DPPIV gene. This increased expression allows for a greater level of DPPIV enzyme in the enterocytes, promoting the more thorough breakdown of any exorphins produced by the proteases.

The second and equally interesting possibility is that galactose is serving as a fuel source of the beneficial microflora (i.e., probiotics) in the gut. This is important because the probiotic organisms themselves contain enzymes capable of breaking-down such exorphins. Varmanen et al (2000, pp. 146-54) recently showed that probiotic organisms, some of which are currently utilized as health supplements, contain analogs of the Dipeptidyl peptidase IV enzyme (e.g., PepX^12), which is thought to be able to digest the theorized exorphins. With over 10^11 microorganisms in the gut, their contribution of enzymatic activity may far exceed that of the enterocytes. It is well documented that galactose is a prebiotic (i.e., stimulates growth of probiotics) and can increase the number of probiotics in the gut.15-16 The significance of this will be expanded upon below.

It is theorized here that what is happening is both the level of DPPIV in the gut is increased and the level of DPPIV-type activity, due to a large increase in the bacterial flora, is also increased. The net result is that due to the rapid formation of any exorphins, from the high level of acid stable protease, the levels of absorbed exorphins dropped below a threshold required for manifestation of the parameters measured. To test this hypothesis, further studies are planned to assay urinary polypeptide levels on retained samples both before and after treatment.17 Also, future studies will look at blood peptide levels both before and after treatment.

Additionally, further studies are planned to determine the genommeptic contribution to the formula. Again,
increasing levels of galactose will be assessed against all the parameters measured in the present study, along with blood levels of exorphins and stool levels of various probiotic species.

The only known contraindication for the use of galactose is in a fairly rare metabolic disorder, galactosemia. However, the amount of galactose in the formula, 100 mg, is far less than that present upon full digestion of a glass of milk. A typical glass of milk contains around 12 grams of lactose, which, when broken down, would contribute six grams of galactose to the diet. That is approximately 60 times the amount in a capsule of ENZYME-ED.

As an uncontrolled pilot clinical study the present work has tremendous value. Not only did the overwhelming majority of parameters measured show a significant benefit from the enzyme blend, but there were also very few associated negative reactions. Also, this is the first time a genomics approach has been therapeutically applied, not only to autism, but to any disease state. While the etiology of autism and the ASD's still remains elusive, clearly the present study advances the knowledge base for efficacious treatment protocols.

Additionally, the present work adds to our body of knowledge that certain probiotics may be a viable complement and possible alternative to fungal based enzymes. Further noted is the 'sideactivity' of many of the enzymes used. While a particular enzyme may be classified as a carbohydrate of some sort, such as lactase, it is well-known that many if not all enzymes also have other targets than their primary one that classifies them. That is to say, given that lactase digests the milk sugar lactose, a disaccharide, it may also digest, albeit not as well, other disaccharides. We know the kinetics of enzymatic activity is that a certain portion of the enzymes present will have their active sites bound, at any given time, by other materials that can fit in the site. The degree to which that happens depends on the enzyme. However it is also known there may be more than one 'catalytic' domain to an enzyme. It is usual to think of an enzyme-substrate complex as being 1:1. One enzyme, one substrate, and that is all. However, that is not the case in vivo.

It is crucial to remember these molecules are constantly moving around and vibrating both in solution and in the cell. They don't sit still like a lock in the door does (remember, we have members, not cell-walls, and hence the fluid mosaic model of cells-they are constantly moving), waiting for a key (though that is the mechanism and visual often presented). The degree to which that is done is enzyme specific and certainly not fully characterized for all the fungal enzymes presently available at the commercial level. It would be interesting to check a formula such as this, for any other disaccharides activity, which may be of interest in the area of autism.

The next few years should reveal some exciting information as to the efficacy and more thorough characterization of enzymes both from fungal sources as well as from probiotic sources. Hopefully, the present study will motivate others to do similar studies, as our body of knowledge grows, with other novel enzyme preparations. There are thousands of enzymes present in any given probiotic species and currently only a few dozen commercial enzymes from fungi are supplied. Imagine the potential when we tap into the probiotics, perhaps with a Genomoeconomical approach to increase expression of the desired enzymes! It is truly staggering.

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References

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