THESIS PROPOSAL

A POTENTIAL DIETARY-BASED ENVIRONMENTAL TRIGGER FOR

HUNTINGTON'S DISEASE

Submitted by

Trevor Brian Connor

Department of Health and Exercise Science

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2010

COLORADO STATE UNIVERSITY

May 1, 2010

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR

SUPERVISION BY TREVOR CONNOR ENTITLED A POTENTIAL DIETARY-

BASED ENVIRONMENTAL TRIGGER FOR HUNTINGTON'S DISEASE

BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE.

Committee on Graduate Work

_____

TBD

_____

TBD

_____

Advisor, Dr. Loren Cordain, Ph.D.

_____

Department Head, Dr. Gay Israel, Ph.D.

Spring 2010

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Huntington's Chorea or Huntington's Disease is a neurodegenerative condition that causes a gradual deterioration of neurons in the striatum and cortex regions of the brain. The disease affects 70 out of every 1 million people of Western European decent. Symptoms include loss of motor function, loss of cognitive function, and chorea (involuntary movements.) Symptoms generally begin when patients are in their 40s or 50s but in rare cases can appear as early as 20 years of age. Once symptomology starts, life-expectancy is reduced to 20 years or less. It is not the disease that causes death however, but most commonly an infection resulting from a loss of immunocompetence associated with the illness (Leblhuber, et al., 1998).

While Huntington's shares many symptoms and pathological mechanisms with other neurodegenerative diseases (NDD) such as Parkinson's Disease and Alzheimer's Disease, it is the only NDD with a known genetic component that exists in all cases of the disease. Huntington's has an autosomal dominant inheritance. If one parent has the condition, a child has a 50% chance of inheriting it (Leblhuber, et al., 1998). Because of the genetic nature of the disease, a certain inevitability has been associated with Huntington's. If a person inherits the requisite allele, prognosis is considered a certainty.

The fact that there appears to be a perfect correlation between genetics and disease manifestation has resulted in very few researchers considering the possibility of an environmental trigger for the Huntington's. Recently however, a case study has brought the genetic certainty of the disease's progression to question. An anonymous woman diagnosed with Huntington's enrolled in a *Crossfit* course and started a wheat-free Paleo Diet recommended by the program. Within six months of starting the diet, her lesions and symptoms disappeared. This reversal of disease progression is virtually unheard of and has raised the question of a potential environmental factor in Huntington's.

Statement of Problem

The purpose of this study is to identify a potential pathological pathway for Huntington's Chorea that includes a dietary environmental trigger. In this case, the possibility of a wheat-based diet as the trigger will be explored.

Primary Hypothesis

It is proposed that Huntington's Disease has both an already identified genetic component and a yet to be identified diet-based environmental trigger.

Secondary Hypotheses

1. Gliadin, from a wheat-based diet, is the environmental trigger.
2. The trigger acts through the binding and up-regulation of tissue transglutaminase, an enzyme central to the pathogenesis of HD

The Null Hypothesis

There is no environmental trigger for HD. Disease progression is determined by genetic susceptibility.

CHAPTER II

LITERATURE REVIEW

Huntington's Disease and Mutant Huntingtin Protein

Huntington's Disease (HD) is an autosomal dominant genetic disorder characterized by extensive neuronal loss in the striatum and cerebral cortex (Wilhelmus, van Dam, & Drukarch, 2008). The severity of symptoms has been directly correlated with the degree of neuronal loss (Hoffner & Djian, 2005). Patients generally do not become symptomatic until their 40's or 50's, though in rare cases symptoms have been known to begin around the age of 20 (Leblhuber, et al., 1998). While cognitive and physical symptoms such as dementia and ataxia have been traced to the graded loss of neurons in the basal ganglia (White, et al., 1997), infection is usually the cause of death in patients who have become severely disabled (Leblhuber, et al., 1998).

Current research has advocated inhibitors such as cystamine as a potential method for treating HD. Cystamine inhibits both tissue transglutaminase (Wilhelmus, et al., 2008) and several caspases which are known to be involved in neuronal cell apoptosis (Lesort, Tucholski, Miller, & Johnson, 2000). However, effective treatment for the disease is still limited. If a patient has the Huntington's gene mutation, a child has a 50% chance of inheriting the disease and once symptomology begins, life-expectancy is reduced to 20 years or less (Wilhelmus, et al., 2008).

Unlike other neurodegenerative diseases such as Parkinson's Disease (PD) and Alzheimer's Disease (AD), which are known to have both hereditary and sporadic manifestations (Bushara, Goebel, Shill, Goldfarb, & Hallett, 2001), the genetic component is always present in Huntington's Disease. The genetic sequence associated with Huntington's Disease is located on chromosome 4 and codes for huntingtin protein (hTT) which is an approximately 350-kD protein (Cummings & Zoghbi, 2000; White, et al., 1997). The disease can be traced to the elongation of a normally polymorphic CAG trinucleotide repeat within the gene sequence (Trottier, et al., 1995). The CAG repeats lengthen a stretch of glutamine residues near huntingtin's amino terminus (White, et al., 1997). The normal repetition range is anywhere from 6 to 35 units. The threshold for Huntington's Disease is 37 to 40 glutamines (Trottier, et al., 1995). Severity of the disease has been directly linked to the number of repeats with ranges as high as 100 repeats being seen in severe cases of the disease (White, et al., 1997). While the damage associated with HD is limited to neuronal tissue in the brain, hTT is present in tissues throughout the body (Hoffner, et al., 2005).

There is some evidence that the lengthened form of hTT, generally referred to as mutant huntingtin protein (mhTT), undergoes a conformational change once a minimum number of repeats is achieved. This change appears to be highly length dependent demonstrated by the fact that the length of the repeat increased the affinity for the mutated protein by a specific IC2 antibody to mHTT (Trottier, et al., 1995). Interestingly, the lengthening of the protein does not appear to affect its stability, cytoplasmic intracellular location, or its expression in adult brains (Aronin, et al., 1995; White, et al., 1997).

While the exact function of huntingtin protein is still being determined, it appears to have a very important role in development. In knockout studies with mice, over 50% of mice with homozygose genes for reduced hTT expression were either stillborn or died within a few days. Those that did survive had extremely abnormal development of the central nervous system including misshapen fore and midbrain regions, ectopic tissue development in the striatum and architectural abnormalities in the striatum (White, et al., 1997). When a 50 CAG repeat allele for hTT was reintroduced, the abnormalities disappeared indicating that mhTT does not impair normal development (White, et al., 1997).

The fact that mhTT does not interfere with normal development has led researchers to hypothesize that mhTT's role in Huntington's Disease involves a gain and not a loss of function in the protein (Hoffner, et al., 2005). Further evidence indicates that mhTT's role in Huntington's Disease may be indirect since huntingtin is expressed more in large cholinergic neurons of the striatum which are relatively spared during the progression of Huntington's Disease (Ferrante, Beal, Kowall, Richardson, & Martin, 1987).

Protein Aggregation

A hallmark of many neurological diseases, including Huntington's, Parkinson's, and Alzheimer's is the development of pathological lesions containing both intra and/or extracellular accumulations of misfolded proteins called *aggregates* (De Vivo, et al., 2008a; Wilhelmus, et al., 2008). While the specific proteins in each disease differ, what is common is the self-interaction of these proteins to form neurotoxic depositions in specific neuronal tissue (Bailey & Johnson, 2005; Wilhelmus, et al., 2008). It is important to note

that while the huntingtin protein is mutated in all case of HD, the proteins involved in AD

and PD have mutated polyglutamine tracts only in non-sparodic forms of these two

diseases (Wilhelmus, et al., 2008).
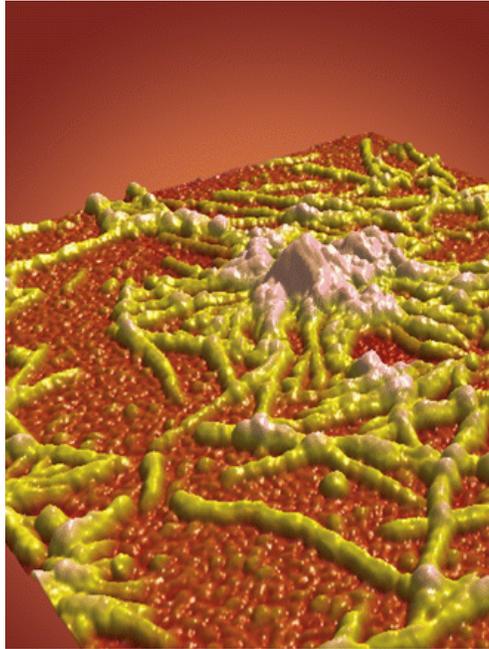
Figure 1. Atomic force microscopic image of huntingtin aggregation. Source: (Legleiter, et al., 2009).

The exact pathway for aggregate formation and the degree of toxicity of the

aggregates vary. The most common aggregation pathway starts when the proteins interact

with themselves to form dimers and trimers. Eventually these dimers and trimers merge

to create oligomers which are characterized by Beta-sheet formation (Wilhelmus, et al.,

2008). Soluble protofibrils are also produced which convert to insoluble mature fibrils

that deposit in the specific areas of the brain. These deposits result in the structures which

are characteristic of neurological diseases (Wilhelmus, et al., 2008). It is believed that the

smaller oligomers are the most neurotoxic since their presence is directly link to

cognitive decline (Harper & Lansbury, 1997).

In Huntington's Disease, aggregation has been linked to the disease's progression. Inclusions containing aggregated mhTT have been found in the regions of the brain affected by HD (Hoffner, et al., 2005). There is a direct correlation between the frequency of these inclusions and the severity of the disease (Becher, et al., 1998). Further, the over-expression of heat shock proteins which are known to reduce inclusions have been shown to reduce the rate of neuronal cell death (Chai, Koppenhafer, Bonini, & Paulson, 1999).

While the correlation between mhTT aggregation and disease progression has been well established, a question that still remains is what causes the aggregation of mutant huntingtin in the first place. It has been proposed that the polyglutamine expansion in mHTT increases its propensity to self-interact and form β-sheet rich aggregates (Wilhelmus, et al., 2008) implying that mhTT initiates the aggregation process itself. However, mhTT is expressed in all stages of both development and adulthood in genetically susceptible individuals. Yet, the disease has a late onset, and no aggregation was found in the brains of pre-symptomatic adults who died of accidental causes (Hoffner, et al., 2005). If the aggregation of mhTT was self-initiating, it would be assumed that the aggregation process would begin at a much younger age. Something has to initiate the self-interaction of these proteins which then leads to the aggregation cascade. One theory is that the process is started when mhTT interacts with another protein. One protein in particular, tissue transglutaminase, has been identified as a potential initiator (Wilhelmus, et al., 2008).

Tissue Transglutaminase

Transglutaminase (TG) is a family of calcium dependent enzymes. There are a total of eight TGs in the human body (De Vivo, et al., 2008a) but only three variations are expressed in the brain. They include tissue transglutaminase (tTG), TG1, and TG3 (Kim, Grant, Lee, Pant, & Steinert, 1999). Tissue transglutaminase is the most abundant of the transglutaminases. It is ubiquitously distributed and is found in all tissues of the body. Once formed, it has a relatively short existence with a half-life of only 11 hours (Wilhelmus, et al., 2008).

Eighty percent of the TG in the body is located in the cytosol (Martin, et al., 2006), however, most of the inclusions associated with HD are located in the nuclei. In a study of neuroblastoma cells, the nuclei contained only 7% of the TG of the cell but accounted for 50% of the total activity. When intracellular calcium levels were increased, such as during apoptosis, some active TG translocated to the nucleus (Hoffner, et al., 2005).

In tTG, the cysteine (Cys) active site is located at the C-terminal domain. Calcium must bind with the enzyme to cause a conformational change to reveal the active site before tTG can perform its enzymatic activity (Ambrus, et al., 2001). Phospholipids are known to up-regulate the sensitivity of tTG to calcium which may account for why a great deal of tTG is located along the nuclear and cell membranes (Lai, Bielawska, Peoples, Hannun, & Greenberg, 1997). While calcium up-regulates the activity of tTG, GTP is a known inhibitor. The two compete for binding to tTG, regulating its activity (Martin, et al., 2006).

The main function of tissue transglutaminase is to irreversibly modify proteins post translation. It catalyzes several reactions, all of which require glutamine as its primary

substrate and another molecule or residue, most frequently an amine, as the secondary substrate (De Vivo, et al., 2008a). The first reaction that tTG catalyzes is the formation of a (γ-glutamyl)polyamine bond. However, as a second reaction, tTG will sometimes preferentially react with H_2O over an amine resulting in a deamination of the glutamine residue (Hadjivassiliou, Aeschlimann, Strigun, Sanders, Woodroofe, & Aeschlimann, 2008). Potentially the most important reaction for tTG in terms of the pathogenesis of Huntington's Disease is the formation of covalent ε-(γ-glutamyl)lysine isopeptide bonds between glutamine and lysine forming both intra and intermolecular cross-links (Wilhelmus, et al., 2008). These cross-links change the conformation of glutamine containing proteins making them more prone to self-interact and aggregate. Interestingly, these cross-links induce stable, rigid and insoluble structures very similar to the deposits found in the inclusions of neurological diseases (Wilhelmus, et al., 2008).

The cross-linking of glutamine to the amine is a two-step process. First, Calcium binds to tTG exposing the active cysteine residue site which then binds with a glutamine residue to form a thioacyl-enzyme intermediary product and ammonia. In the second step, the intermediary product reacts with a nucleophilic amine and cross-links the amine-containing donor to the glutamine residue (De Vivo, et al., 2008a; Wilhelmus, et al., 2008).

The intermediary thioacyl-enzyme of this reaction has become highly relevant to the research of both Huntington's disease and multiple chronic illnesses. Recent research has pointed towards this intermediary product between tTG and gliadin as the potential auto-immune antigen in celiac disease and several other gluten-related autoimmune illnesses (Gorgun, Portyanko, Marakhouski, & Cherstvoy, 2009).

Figure2. Tissue transglutaminase catalyzes the two step reaction between a glutamine residue and lysine. Source: (De Vivo & Gentile, 2008a).

Tissue transglutaminase appears to also have a role as a G protein, but it is believed that this role is not associated with neurological disorders (Nakaoka, et al., 1994).

The exact function of tTG in the human body is still relatively unknown. Initial knockout studies in rats produced no effect on phenotypical development (De Laurenzi & Melino, 2001; Martin, et al., 2006). However, closer examination in later studies demonstrated subtle but important roles in cell apoptosis and some immune function (Sarang, et al., 2009). tTG enhanced the apoptosis of thymocytes and erythrocytes. It aided macrophage digestion of apoptotic cells, and played a critical role in the proper differentiation and bacterial killing of neutrophils (Sarang, et al., 2009). These findings led Sarang et. al. (2009) to conclude that the main role of tTG was "to ensure that once

apoptosis has been initiated, it is finished without causing inflammation and apparent tissue injury."

Other potential roles of tTG include growth of neuritis (Maccioni & Seeds, 1986), cell differentiation, matrix stabilization, and cell adhesion (Reif & Lerner, 2004). In these last two roles, it is believed that tissue transglutaminase cross-links cell matrix proteins, strengthening them to aid cell adhesion and motility. This ability to cross-link extraceullular matrices is critical during both the healing of cellular damage and during apoptosis since it increases cellular stability and prevents inflammation or the release of cellular contents (Martin, et al., 2006; Sarang, et al., 2009).

While much of the attention on tTG has been directed at its role in apoptosis, it is believed that tTG also plays a role in the development of the nervous system since its levels and activity are dramatically increased during the brain's growth spurt (Bailey & Johnson, 2004).

It is important to point out that because of the need for sufficient calcium to bind tTG, it's activity is latent under normal physiological conditions (Martin, et al., 2006). It is only under pathological conditions, such as cell death, that sufficient calcium is released to activate the enzyme. This has led some researchers to conclude that tTG activation may be a consequence of apoptosis and not the cause (Hoffner, et al., 2005).

Tissue Transglutaminase and Its Role in mHTT Aggregation

The self-interacting proteins of AD, PD, and HD are all known substrates of tTG and increased levels of tTG activity have been observed in almost all neurodegenerative diseases (Wilhelmus, et al., 2008). In HD, the level of tissue transglutaminase activity and expression were strongly correlated with disease severity (Lesort, Chun, Johnson, &

Ferrante, 1999). Further, tissue transglutaminase cross-links were elevated in the cerebral spinal fluid of HD patients and these cross-links were also found to colocalize with mhTT in the intranuclear inclusions associated with HD (Zainelli, Ross, Troncoso, & Muma, 2003). In a study where the genes for tTG were knocked out in Huntington's positive R6/2 transgenic mice, lifespan was extended 19.7% compared to mice with the tTG alleles, and disease severity and onset were less severe (Bailey, et al., 2005). While it is relevant that removal of the tTG alleles did not completely prevent HD onset in the mice, it is important to point out that the total TG activity in the mice was not eliminated. However activity was reduced to 26% of that in wild-type mice indicating tTG is responsible for the majority of TG activity (Bailey, et al., 2005).

Similar to tTG knockout studies, the introduction of cystamine, which acts as a TG inhibitor, but also inhibits some of the crucial initial steps of apoptosis, was shown to decrease the number of striatal huntingtin protein aggregates in HD positive mice and reduce disease severity (Bailey, et al., 2005)

As was noted above, glutamine residues are the primary substrate of tTG. This is relevant because mutant huntingtin is characterized by excessive repeats of glutamine residues. It has been found that a polyglutamine segement acts as an excellent substrate for tTG as long as the residues on either side render the peptides sufficiently soluble. Lengthening the polyglyutamine tract not only increases their solubility, but also increases the reactivity of each glutamine residue with one another (Hoffner, et al., 2005). It has been proposed that increasing the glutamine repeats beyond a certain threshold allows the mutated huntingtin protein to become a substrate for tTG (Lesort, et al., 1999). *In viro* studies have confirmed that the lengthened huntingtin protein acts as a substrate

for tTG and the rate constant of the reaction increased significantly with the number of repeats (Hoffner, et al., 2005).

Tissue transglutaminase has been implicated as the initiator of the aggregation of mhTT. By cross-linking huntingtin proteins, tTG could stimulate the aggregation process (Wilhelmus, et al., 2008). This fact has led many researchers to conclude that tissue transglutaminase, in the presence of calcium, may serve as a key regulator of huntingtin aggregation in HD disease progression (Hoffner, et al., 2005; Martin, et al., 2006; Wilhelmus, et al., 2008).

While the bulk of current research points to a key role for tTG in HD pathogenesis, some contradictory research does exist. First, TG activity is increased in the cerebellum where Huntington's does not manifest. Further, tTG is a predominantly cytoplasmic enzyme, while the inclusions associated with HD are frequently nuclear (Hoffner, et al., 2005). Though, as was pointed out before, elevated calcium levels do cause a translocation of tTG into the cell nucleus (Hoffner, et al., 2005). Finally it has also been shown that polyglutamine tracts can self-aggregate *in vitro* in the absence of tTG through the formation of anti-parallel β-sheets called 'polar-zippers' (Bailey, et al., 2005; Burke, Woscholski, & Yaliraki, 2003).

Gliadin and the Gut Barrier

Under normal physiological conditions, the digestive system provides a series of defensive mechanisms to prevent the entry of toxic or potentially harmful organisms and macromolecules into the internal system. The healthy gut mucosa along with its brush border and tight junctions are designed as the main barrier to passage of any macromolecules or pathogens (Visser, Rozing, Sapone, Lammers, & Fasano, 2009). Most

digested materials are broken down into their basic monomers before passing the gut

barrier. Only about 2% of intact dietary proteins are allowed passage (Cordain, Toohey,

Smith, & Hickey, 2000).

Under normal conditions, the gut barrier is designed to block entry of any ingested

pathogens or pathogenic triggers. So, for a dietary trigger of Huntington's Disease to

exist, one of two conditions must occur. Either the relevant dietary compound must

escape detection as a potentially damaging foreign pathogen, or it must find a way to get

past the gut barrier defenses. Almost all digested proteins are broken down into basic

amino acids before passing the gut barrier. Those digested macromolecules that do pass

the barrier are allowed entry so that they can be presented to the immune system by

antigen presenting cells (Visser, et al., 2009). So, it is highly unlikely that a dietary

pathogenic trigger could simply pass the gut barrier intact and undetected. More likely a

flaw or weakening of the defensive system allows entry of the suspected trigger.

Recently, gliadin which is a part of the gluten family of proteins found in wheat,

barley, rye and possibly oats, has gained attention as a dietary trigger for multiple

disorders including celiac disease (Bushara, Nance, & Gomez, 2004) and psoriasis

(Michaelsson, Ahs, Hammarstrom, Lundin, & Hagforsen, 2003). Like huntingtin protein,

gliadin contains a polyglutamine tract that is a known substrate for tTG (Roth, et al.,

2008). To exert its pathogenic effects, gliadin must enter the subepithelial layer from the

intestinal lumen to interact with the gut-associated lymphoid tissue (GALT) and stimulate

autoimmune processes (Drago, et al., 2006; Visser, et al., 2009).

Gliadin is able to both avoid digestive breakdown and pass the gut barrier intact. The

digestive system contain proteases designed to break down digested proteins to smaller

peptides and basic amino acids before they are absorbed through the epithelial border of the digestive system. Unlike most proteins, gliadin and wheat-germ agglutinin (WGA), another wheat-based protein, are resistant to digestive proteolytic breakdown (Brady, Vannier, & Banwell, 1978; Cordain, et al., 2000).

The first line of defense of the epithelial layer to the external contents of the digestive tract is the brush border. This border which lines the entire surface of the intestinal tract is composed of thin hair-like structures called microvilli which protrude from the apical surface of intestinal epithelial cells (Cordain, et al., 2000). Several classes of molecules can break down the brush border and allow intestinal contents direct access to intestinal epithelial cells. These include saponins, a triterpenoid glycosides capable of damaging villi (Francis, Kerem, Makkar, & Becker, 2002), lectins, a sugar-binding protein, and WGA. Lectins and WGA act by binding to surface glycans and damaging the base of the villi. This damaging action includes weakening of the cytoskeleton, increased endocytosis and a shortening of the microvilli (Cordain, et al., 2000; Pusztai, et al., 1993). These structural changes by WGA, lectins, and saponins, increase digestive permeability allowing gliadin and other undegraded dietary antigens to gain access to the epithelial layer and ultimately to systemic circulation (Cordain, et al., 2000).

Two pathways have been identified for gliadin's entry into circulation once it has passed the brush border; transcellular transport and paracellular permeability (Clemente, et al., 2003; Drago, et al., 2006). While the two do not need to be mutually exclusive, the fact that heightened paracellular permeability proceeds the onset of celiac disease symptoms (an autoimmune disease associated with gliadin entry) indicates that paracellular permeability may be the primary pathway (Drago, et al., 2006).

16

Gliadin stimulates paracellular permeability by binding with CXCR3 receptors on the intestinal lumen. This binding causes the release of the protein zonulin (Visser, et al., 2009). The role of gliadin in zonulin release is significant since no other CXCR3 ligand appears to regulate zonulin levels.

Zonulin is a peptide of the digestive system involved in the control of tight junctions between lumen epithelial cells (Drago, et al., 2006). The levels of zonulin vary in the digestive system with the highest concentrations found in the jejunum and distal ileum. It has not been found in the colon and it's levels decrease along the callous-crypt axis (Fasano, Uzzau, Fiore, & Margaretten, 1997). Zonulin is known to play a role in multiple autoimmune pathogeneses with zonulin-regulated permeability preceding the onset of both Type I Diabetes and Celiac Disease (Drago, et al., 2006).

Actin microfilaments exist throughout the tight junctions forming a cytoskeleton that binds the epithelial cells and regulates paracellular passage of digested particles in the healthy lumen (Visser, et al., 2009). Zonulin causes a protein kinase C-mediated polymerization of these actin filaments, reorganizing the cytoskeleton and allowing the junctions to open and increase permeability (Drago, et al., 2006). ZO-1 is a key component of this junctional complex. Flourescent imaging of intestinal epithelial cells *in vitro* can detect the presence of the ZO-1 protein along the junctional borders. In one study, a fluorescent irregular pattern was shown soon after exposure to gliadin suggesting tight junction disassembly. Interestingly, the effect had completely reversed two hours after gliadin removal indicating that the gliadin-zonulin effect on tight junctions is rapid and reversible (Drago, et al., 2006).

It is important to point out that tight junction dysfunction is not limited to CD pathogenesis. It has been found in multiple conditions including food allergies, infections of the GI tract, autoimmune diseases (Visser, et al., 2009), and inflammatory bowel diseases (Fasano, 2001). Serum zonulin concentrations were significantly higher ($P<0.000001$) in the acute phases of both Celiac Disease and Type I Diabetes. These levels were reduced on a gluten-free diet (Fasano, et al., 2000). Equally as important, this tight-junction mediated response is not even unique to disease states. In a study conducted by Drago et. al. (2006), PT-gliadin exposure caused an up-regulation of zonulin and tight junction actin changes in both celiac and healthy subjects. The release of zonulin in celiac patients was simply more rapid (5 minutes verses 15 minutes), lasted longer (greater than an hour verses 30 minutes) and the tight junction changes reached significance faster (15 minutes verses 60 minutes) in the celiac patients. Unlike the healthy subjects however, once gliadin was recognized by intestinal T cells in celiac patients, cytokines and matrix metalloproteinases were up-regulated causing further damage to the intestinal mucosa (Molberg, et al., 2001).

The negative impact of gliadin crossing the gastrointestinal barrier may be magnified by the simultaneous uptake of luminal bacteria once tight junctions have been opened (Cordain, et al., 2000). In HLA-B27 transgenic rats with arthritis, both gut inflammation and joint inflammation were eliminated when the rats were raised in a germ-free environment. These results indicated a potential connection between gut permeability, intestinal antigens, and inflammatory disease (Taurog, et al., 1994). Changes in the balance between beneficial and harmful intestinal bacteria have since been associated

with several disorders including allergies, Type 1 Diabetes, and several inflammatory bowel diseases (Visser, et al., 2009).

Gliadin-Transglutaminase Interaction and the Immune Response

Once gliadin has crossed the epithelial layer of the digestive system it is exposed to two possible processes. First, it is exposed to a variety of immune processes in the GALT. Second, it can potentially bind to tissue transglutaminase located in the digestive subepithelium.

Gliadin has a large number of glutamine residues that have been shown to act as substrates for tTG *in vitro* (Porta, Esposito, Gentile, Mariniello, Peluso, & Metafora, 1990). The binding of gliadin to tTG is a known step in the pathogenesis of celiac disease (Gorgun, et al., 2009). Tissue transglutaminase levels are generally up-regulated in the duodenal mucosa of celica patients, particularly in the lamina propria (Gorgun, et al., 2009). However, this up-regulation appears to be a response to a secondary process and not a direct result of the disease since levels were reduced in patients on a gluten-free diet. Potential mitigating factors include mucosal atrophy, inflammation, or gluten intake (Gorgun, et al., 2009).

It is believed that tTG catalyzes an atypical deamination of gliadin's glutamine residues in CD patients (Roth, et al., 2008). Some current research contends that the reaction between tTG and gliadin is specific to celiac patients. Tissue transglutaminase antibodies are almost exclusively found in CD, to the point that some authors content that their identification can be used as a positive test for celiac disease (Hadjivassiliou, et al., 2008).

However, other recent studies have found intestinal mucosal levels of tTG to be elevated in multiple inflammatory diseases and not just CD (De Vivo, Martin, Trotta, & Gentile, 2008b). Studies of intestinal pathologies such as Crohn's disease and ulcerative colitis demonstrated a strong correlation between transglutaminase activity and disease severity (D'Argenio, et al., 1995). The changes in tTG levels in inflammatory bowel diseases were in fact very similar to CD; expressing in the epithelium and lamina propria (Gorgun, et al., 2009). It is believed that tTG activates nuclear factor-kappaB which participates in the regulation of inflammation (Lee, et al., 2004) and is necessary for mucosal healing in chronically damaged colons (D'Argenio, et al., 2005).

The up-regulation of tTG and reaction to gliadin also does not appear to be limited to inflammatory bowel conditions. In a study of psoriasis patients, tTG was strongly overexpressed in skin lesions compared to healthy skin. The patients also had elevated antibodies (both IgA and IgG) to gliadin. When placed on a gluten-free diet, both tTG levels and the size and number of skin lesions were reduced (Michaelsson, et al., 2003).

While these studies have demonstrated a correlation between mucosal tTG levels and multiple inflammatory conditions, they do not definitively answer whether tTG plays a causal role in pathogenesis or if its up-regulation is simply a response to damage resulting from chronic inflammation. Only in one condition, celiac disease, has a causal relationship been identified. While tissue transglutaminase is itself a target of auto-antibodies in CD (Michaelsson, et al., 2003), tTG also cross-links itself with gliadin to form an immunoreactive complex (Fleckenstein, Qiao, Larsen, Jung, Roepstorff, & Sollid, 2004; Gorgun, et al., 2009). In the case of CD, this complex is highly reactive with HLA-DQ2/DQ8 (Hadjivassiliou, et al., 2008). The tTG-gliadin complex may

mitigate T-cell sensitivity in CD in one of two ways. First, tTG and deaminated gliadin may share epitopes that result in molecular mimicry (Hadjivassiliou, et al., 2008). Second, the binding of tTG and gliadin may cause a conformational change exposing epitopes that are more reactive with HLA-DQ2/DQ8 (Pinkas, Strop, Brunger, & Khosla, 2007).

The immune response to the tTG-gliadin complex and the human leukocyte antigen (HLA) system are intimately connected (Visser, et al., 2009). HLA genes are the most polymorphic genes in the human body. They exist on the short arm of chromosome 6 and are divided into two classes: class I (HLA-A, HLA-B, HLA-C) and class II (HLA-DR, HLA-DQ, HLA-DP) (Cordain, et al., 2000). These genes code for glycoprotein receptors found in antigen presenting cells (APC) that present antigens to the T cell system in the intestinal mucosa (Bjorkman, Saper, Samraoui, Bennett, Strominger, & Wiley, 1987). Specific HLA variations are associated with over 50 diseases including CD and Type 1 Diabetes. In the case of CD, the HLA variants HLA-DQ2 and HLA-DQ8 are necessary genetic component of the disease (Visser, et al., 2009). When HLA-DQ2 and HLA-DQ8 present epitopes of the tTG-gliadin complex, they trigger a CD4+ T-cell response to gliadin and self-antigens (Hadjivassiliou, et al., 2008) that result in an autoimmune response.

Gluten Ataxia and the Diverse Manifestations of Gluten Sensitivity

Celiac Disease, Huntington's Disease and several neurodegenerative disorders share a common symptom: ataxia or uncontrollable jerky movements. One form of ataxia, called gluten ataxia is associated with gluten sensitivity (Bushara, et al., 2004). It is

commonly linked to CD, but recent studies have shown that gluten ataxia can present

with or without the intestinal pathology seen in CD (Bushara, et al., 2001; Hadjivassiliou,

et al., 2008). Further, the cerebellar degradation and symptomology was indistinguishable

from other ataxias (Bushara, et al., 2001). The dominant antibody in the CNS of patients

with gluten ataxias was to a member of the transglutaminase family: TG6. TG6 was not

found in the CNS of normal subjects (Hadjivassiliou, et al., 2008).

This identification of an autoimmune response resulting from TG6 and gliadin has

led some researchers to believe that the development of autoimmunity directed at the

various proteins of the transglutaminase family may allow gluten sensitivity to take on a

variety of manifestations (Hadjivassiliou, et al., 2008).

Huntington's Disease and Immunity

Huntington's Disease is not considered an autoimmune disease. However, evidence

of a yet to be determined endogenous antigen has been mounting in patients with HD

(Bushara, et al., 2004). This endogenous antigen is not proof positive of an autoimmune

component though since these immune markers could simply indicate the presence of a

low level secondary infection (Leblhuber, et al., 1998).

One study demonstrated a higher than normal level of antigliadin antibody (AGA) in

44.2 % of Huntington's patients compared to healthy controls (p<0.00001). However HD

patients with and without elevated ADA levels demonstrated no difference based on age

of onset, disease length, or CGA repeats indicating that the higher than normal AGA

levels may be a parallel phenomenon (Bushara, et al., 2004).

In another study, a specific immune response to HD brain tissue was demonstrated.

Lympohcytes from Huntington's patients responded to HD brain tissue *in vitro* but not to

tissue from normal, AD, or PD brain tissue. Further, lymphocytes from non-HD patients did not respond to the HD tissue (Barkley, Hardiwidjaja, & Menkes, 1977).

In a more recent study, neopterin levels were tested in the spinal fluid of HD patientis. Neopterin is a product of immunocompetent cells such as macrophages and T-cells and is an indicator of activated cellular immunity. Neopterin levels were elevated in HD patients ($t=6.55$, $P<0.001$) (Leblhuber, et al., 1998).

Interestingly, the immune markers in HD are very similar to those found in AD and many autoimmune and inflammatory diseases (Leblhuber, et al., 1998).

CHAPTER III

METHODS AND PROCEDURES

Procedures

The focus of this thesis project will be a thorough review of current research on

Huntington's Disease, transglutaminase, and potential dietary mechanisms of the disease.

The existing literature has made great strides in describing the processes occurring both

at the neural and digestive levels. What has yet to be described is the connection between

these processes. It is the hope that this thesis will add that connection to the existing body

of research. In order to address these connections and the hypothesis of this thesis,

several questions must be answered. The following sections identify several of the

questions that will be addressed and summarizes some current research that may point to

the potential answer for each question.

*Question 1: What Evidence is There of an Environmental Trigger in HD?*

Huntington's Disease is a late onset condition. Symptomology does not appear until

a patient is in their late 30s or early 40s. Earliest onset is seen around 20 years of age and

is considered extremely rare (Leblhuber, et al., 1998). The genetic component of the

disease has been linked to an expanded polyglutamine tract in huntingtin protein (White,

et al., 1997). So, if the disease were entirely genetic with no environmental trigger, it is

reasonable to postulate that mhTT either loses its function or does not become active until later in life.

It has already been demonstrated that huntingtin protein is essential to development and mhTT still fulfills this developmental role. In a study of mice with the gene for either mutant huntingtin or normal huntingtin knocked out, the majority of mice were stillborn and those that did survive experienced extreme developmental abnormalities. Reintroduction of even a 50 CAG repeat mhTT restored normal development (White, et al., 1997). So, the role of mhTT in Huntington's Disease does not appear to be due to a loss of function.

If the disease were entirely genetic with no trigger or secondary factor, then it could be hypothesized that aggregation and disease symptoms would begin with the expression or activation of mhTT. This again does not appear to be the case. mhTT and hTT express comparably in development and adulthood suggesting that both are regulated similarly (Aronin, et al., 1995). Further in studies of hTT and mhTT activity in rat brains, both were shown to have their highest level of expression and activity in early development – at a time when Huntington's Disease pathology is not active in the animals (Bhide, et al., 1996).
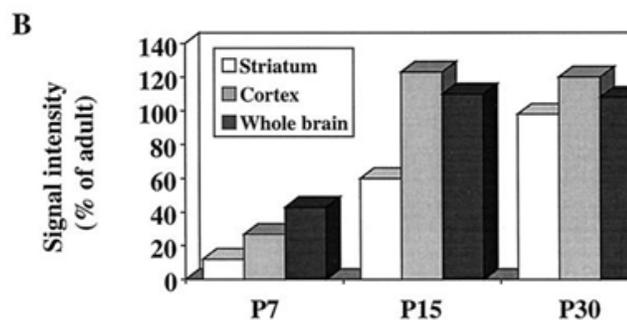
Figure3. Comparison of hTT activity during development to adult levels in HD positive mice. Source: (Bhide, et al., 1996)

25

Some researchers have attributed the late of onset of HD despite the heightened activation of mhTT in development to a gradual aggregation process. Neurotoxic aggregates accumulate from a very early age but do not reach toxic levels until much later (Hoffner, et al., 2005). However, in a study of an adult who carried the gene for HD but died of accidental causes prior to the onset of symptoms, there was no evidence of inclusions in the cortex or striatum (DiFiglia, et al., 1997).

Question 2: Are tTG and mhTT Causal in HD?

It has already been pointed out above that mhTT positive R6/2 mice with tTG knockouts had a 19.7% increase in life expectancy and a significant reduction in disease symptoms (Bailey, et al., 2005). Up-regulation of tissue transglutaminase has even been shown to facilitate neuronal cell apoptosis without any protein aggregation (Tucholski & Johnson, 2002).

While tTG clearly plays a clear role in Huntington's pathogenesis, the correlation between mhTT aggregation and disease progression is interestingly weaker. The same studies found that the number of huntingtin protein aggregates did not correlate with disease progression. Further, in tTG negative transgenic mice, mhTT aggregation was actually increased despite a reduction in disease severity (Bailey, et al., 2005). In another study, the inhibition of huntingtin aggregates actually increased neurotoxicity and neuronal apoptosis (Saudou, Finkbeiner, Devys, & Greenberg, 1998). Even the localization of mhTT aggregates has demonstrated limited correlation to disease progression. mhTT aggregates were found in striatal neurons spaced in HD, while aggregates rarely formed in medium-sized spiny neurons that were lost (Kuemmerle, et

al., 1999). This body of evidence has led many researchers to conclude that instead of being causal in HD, aggregation may actually be protective.

If mhTT aggregation is not causal, then the door is open to consider the idea that tTG and its binding with mutant huntingtin may be the causal mechanism in the pathogenesis in HD. However, for this hypothesis to hold, the fact that tTG negative mice still developed HD (albeit with reduced symptoms) must be explained. The answer could lie in the involvement of other members of the TG family in the disease. tTG negative mice still demonstrated TG activity albeit at 23% of the levels in wild type mice (Bailey, et al., 2005).

Question 3: How does the tTG/gliadin response at the gut activate neurodegeneration in the brain?

Roth et al. (2009) have proposed that the neoantigen in most autoimmune diseases is the Michaelis-Menton intermediary complex between an enzyme and its substrate. This appears to be the case in celiac disease, the one autoimmune illness where the environmental trigger has been identified. The tTG-gliadin complex has been proposed as the antigen in celiacs. Epitopes of the complex are presented to the immune system bound specifically to HLA-DQ2 or HLA-DQ8 which sets off the autoimmune response (Gorgun, et al., 2009). However, the response to these specific HLA moieties appears to be specific to CD.

Interestingly, both gliadin and mhTT contain polyglutamine tracts that are known substrates of tTG. The binding of transglutaminase to the polyglutamines in gliadin has been identified as a potential mechanism in multiple autoimmune illnesses (Martin, et al.,

2006; Michaelsson, et al., 2003). Likewise, the binding of tTG to the polyglutamines in

mhTT has been identified as a potentially critical step in the pathogenesis of HD

(Hoffner, et al., 2005).

While HLA-DQ2/DQ8 activity due to gluten ingestion are specific to CD, in a recent

study of dendritic cells *in vitro*, gliadin produced a strong expression of HLA-DR in cells

from both celiac and healthy control subjects (Rakhimova, Esslinger, Schulze-Krebs,

Hahn, Schuppan, & Dieterich, 2009). In an earlier study, brain tissue from HD, PD, and

Alzheimer's patients were stained for HLA-DR. In all cases, the lesion areas of the brain

were so strongly positive that the staining was visible to the human eye (McGeer, Itagaki,

& McGeer, 1988).

Question 4: If tTG and mhTT are expressed in most tissues of the body, why does

pathogenesis only occur in specific regions of the brain?

In celiac disease, activation of the autoimmune response to tTG-gliadin produces a

systemic reaction. Disease symptomology is experienced in many tissues of the body. In

Huntington's however, degeneration occurs only in the cortex and striatum of the brain.

Yet, mutant huntingtin and tissue transglutaminase are distributed through most tissues

of the body. So, if the tTG-gliadin complex in the digestive system produces a response

to the intermediary complex of mhTT and tTG, why isn't the response systemic?

Tissue transglutaminase is a latent enzyme, meaning that it has no function in non-

pathological conditions (Martin, et al., 2006). tTG must be activated before it can bind

with mhTT or gliadin. tTG is only active when calcium levels are high enough such as

during cell damage or apoptosis (Hoffner, et al., 2005). At the gut, several mechanisms

have been proposed for the activation of tTG including chronic damage due to inflammation (D'Argenio, et al., 2005).

It is conceivable that HD pathogenesis only occurs in the cortex and striatum because those are the only locations where calcium, mhTT, and tTG levels are high enough to bring about their binding. In a 2007 review of neurodegeneration diseases such as Alzheimer's, MS, ALS, and Huntington's Disease it was found that cell cycle control failed in target neurons just prior to symptom onset. Cells lost their ability to effectively regulate sodium and calcium balance leading to an elevated level of intracellular calcium (Harguindey, Reshkin, Orive, Arranz, & Anitua, 2007).

REFERENCES

Ambrus, A., Banyai, I., Weiss, M. S., Hilgenfeld, R., Keresztessy, Z., Muszbek, L., et al. (2001). Calcium binding of transglutaminases: A 43ca nmr study combined with surface polarity analysis. *Journal of Biomolecular Structure and Dynamics, 19*(1), 59-74.

Aronin, N., Chase, K., Young, C., Sapp, E., Schwarz, C., Matta, N., et al. (1995). Cag expansion affects the expression of mutant huntingtin in the huntington's disease brain. *Neuron, 15*(5), 1193-1201.

Bailey, C. D., & Johnson, G. V. (2004). Developmental regulation of tissue transglutaminase in the mouse forebrain. *Journal of Neurochemisty, 91*(6), 1369-1379.

Bailey, C. D. C., & Johnson, G. V. (2005). Tissue transglutaminase contributes to disease progression in the r6/2 huntington's disease mouse model via aggregate-independent mechanisms. [Article]. *Journal of Neurochemistry, 92*(1), 83-92.

Barkley, D. S., Hardiwidjaja, S., & Menkes, J. H. (1977). Huntingtons-disease - delayed-hypersensitivity invitro to human central nervous-system antigens. [Article]. *Science, 195*(4275), 314-316.

Becher, M. W., Kotzuk, J. A., Sharp, A. H., Davies, S. W., Bates, G. P., Price, D. L., et al. (1998). Intranuclear neuronal inclusions in huntington's disease and dentatorubral and pallidoluysian atrophy: Correlation between the density of inclusions and it15 cag triplet repeat length. *Neurobiology of Disease, 4*(6), 387-397.

Bhide, P. G., Day, M., Sapp, E., Schwarz, C., Sheth, A., Kim, J., et al. (1996). Expression of normal and mutant huntingtin in the developing brain. [Article]. *Journal of Neuroscience, 16*(17), 5523-5535.

Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L., & Wiley, D. C. (1987). Structure of the human class i histocompatibility antigen, hla-a2. *Nature, 329*(6139), 506-512.

Brady, P. G., Vannier, A. M., & Banwell, J. G. (1978). Identification of the dietary lectin, wheat germ agglutinin, in human intestinal contents. *Gastroenterology, 75*(2), 236-239.

Burke, M. G., Woscholski, R., & Yaliraki, S. N. (2003). Differential hydrophobicity drives self-assembly in huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America, 100*(24), 13928-13933.

Bushara, K. O., Goebel, S. U., Shill, H., Goldfarb, L. G., & Hallett, M. (2001). Gluten sensitivity in sporadic and hereditary cerebellar ataxia. [Article]. *Annals of Neurology, 49*(4), 540-543.

Bushara, K. O., Nance, M., & Gomez, C. M. (2004). Antigliadin antibodies in huntington's disease. *Neurology, 62*(1), 132-133.

Chai, Y., Koppenhafer, S. L., Bonini, N. M., & Paulson, H. L. (1999). Analysis of the role of heat shock protein (hsp) molecular chaperones in polyglutamine disease. *Journal of Neuroscience, 19*(23), 10338-10347.

Clemente, M. G., De Virgiliis, S., Kang, J. S., Macatagney, R., Musu, M. P., Di Pierro, M. R., et al. (2003). Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. *Gut, 52*(2), 218-223.

Cordain, L., Toohey, L., Smith, M. J., & Hickey, M. S. (2000). Modulation of immune function by dietary lectins in rheumatoid arthritis. *British Journal of Nutrition, 83*(3), 207-217.

Cummings, C. J., & Zoghbi, H. Y. (2000). Trinucleotide repeats: Mechanisms and pathophysiology. *Annual Review of Genomics and Human Genetics, 1*, 281-328.

D'Argenio, G., Biancone, L., Cosenza, V., Della Valle, N., D'Armiento, F. P., Boirivant, M., et al. (1995). Transglutaminases in crohn's disease. *Gut, 37*(5), 690-695.

D'Argenio, G., Calvani, M., Della Valle, N., Cosenza, V., Di Matteo, G., Giorgio, P., et al. (2005). Differential expression of multiple transglutaminases in human colon: Impaired keratinocyte transglutaminase expression in ulcerative colitis. *Gut, 54*(4), 496-502.

De Laurenzi, V., & Melino, G. (2001). Gene disruption of tissue transglutaminase. *Molecular and Cellular Biology, 21*(1), 148-155.

De Vivo, G., & Gentile, V. (2008a). Transglutaminase-catalyzed post-translational modifications of proteins in the nervous system and their possible involvement in neurodegenerative diseases. [Article]. *CNS & Neurological Disorders - Drug Targets, 7*(4), 370-375.

De Vivo, G., Martin, A., Trotta, T., & Gentile, V. (2008b). Role of transglutaminase-catalyzed reactions in the post-translational modifications of proteins responsible for immunological disorders. *Inflamm Allergy Drug Targets, 7*(1), 24-29.

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., et al. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science, 277*(5334), 1990-1993.

Drago, S., El Asmar, R., Di Pierro, M., Clemente, M. G., Tripathi, A., Sapone, A., et al. (2006). Gliadin, zonulin and gut permeability: Effects on celiac and non- celiac intestinal mucosa and intestinal cell lines. *Scandinavian Journal of Gastroenterology, 41*(4), 408-419.

Fasano, A. (2001). Intestinal zonulin: Open sesame! *Gut, 49*(2), 159-162.

Fasano, A., Not, T., Wang, W. L., Uzzau, S., Berti, I., Tommasini, A., et al. (2000). Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet, 355*(9214), 1518-1519.

Fasano, A., Uzzau, S., Fiore, C., & Margaretten, K. (1997). The enterotoxic effect of zonula occludens toxin on rabbit small intestine involves the paracellular pathway. *Gastroenterology, 112*(3), 839-846.

Ferrante, R. J., Beal, M. F., Kowall, N. W., Richardson, E. P., Jr., & Martin, J. B. (1987). Sparing of acetylcholinesterase-containing striatal neurons in huntington's disease. *Brain Research, 411*(1), 162-166.

Fleckenstein, B., Qiao, S. W., Larsen, M. R., Jung, G., Roepstorff, P., & Sollid, L. M. (2004). Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. *J Biol Chem, 279*(17), 17607-17616.

Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: A review. *Br J Nutr, 88*(6), 587-605.

Gorgun, J., Portyanko, A., Marakhouski, Y., & Cherstvoy, E. (2009). Tissue transglutaminase expression in celiac mucosa: An immunohistochemical study. *Virchows Arch*.

Hadjivassiliou, M., Aeschlimann, P., Strigun, A., Sanders, D. S., Woodroofe, N., & Aeschlimann, D. (2008). Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. *Annals of Neurology, 64*(3), 332-343.

Harguindey, S., Reshkin, S. J., Orive, G., Arranz, J. L., & Anitua, E. (2007). Growth and trophic factors, ph and the na+/h+ exchanger in alzheimer's disease, other neurodegenerative diseases and cancer: New therapeutic possibilities and potential dangers. [Review]. *Current Alzheimer Research, 4*(1), 53-65.

Harper, J. D., & Lansbury, P. T., Jr. (1997). Models of amyloid seeding in alzheimer's disease and scrapie: Mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annual Review of Biochemistry, 66*, 385-407.

Hoffner, G., & Djian, P. (2005). Transglutaminase and diseases of the central nervous system. [Review]. *Frontiers in Bioscience, 10*, 3078-3092.

Kim, S. Y., Grant, P., Lee, J. H., Pant, H. C., & Steinert, P. M. (1999). Differential expression of multiple transglutaminases in human brain. Increased expression and cross-linking by transglutaminases 1 and 2 in alzheimer's disease. *Journal of Biological Chemistry, 274*(43), 30715-30721.

Kuemmerle, S., Gutekunst, C. A., Klein, A. M., Li, X. J., Li, S. H., Beal, M. F., et al. (1999). Huntingtin aggregates may not predict neuronal death in huntington's disease. [Article]. *Annals of Neurology, 46*(6), 842-849.

Lai, T. S., Bielawska, A., Peoples, K. A., Hannun, Y. A., & Greenberg, C. S. (1997). Sphingosylphosphocholine reduces the calcium ion requirement for activating tissue transglutaminase. *Journal of Biological Chemistry, 272*(26), 16295-16300.

Leblhuber, F., Walli, J., Jellinger, K., Tilz, G. P., Widner, B., Laccone, F., et al. (1998). Activated immune system in patients with huntington's disease. [Article]. *Clinical Chemistry and Laboratory Medicine, 36*(10), 747-750.

Lee, J., Kim, Y. S., Choi, D. H., Bang, M. S., Han, T. R., Joh, T. H., et al. (2004). Transglutaminase 2 induces nuclear factor-kappab activation via a novel pathway in bv-2 microglia. *J Biol Chem, 279*(51), 53725-53735.

Legleiter, J., Lotz, G. P., Miller, J., Ko, J., Ng, C., Williams, G. L., et al. (2009). Monoclonal antibodies recognize distinct conformational epitopes formed by polyglutamine in a mutant huntingtin fragment. *Journal of Biological Chemistry, 284*(32), 21647-21658.

Lesort, M., Chun, W. J., Johnson, G. V. W., & Ferrante, R. J. (1999). Tissue transglutaminase is increased in huntington's disease brain. [Article]. *Journal of Neurochemistry, 73*(5), 2018-2027.

Lesort, M., Tucholski, J., Miller, M. L., & Johnson, G. V. W. (2000). Tissue transglutaminase: A possible role in neurodegenerative diseases. [Review]. *Progress in Neurobiology, 61*(5), 439-463.

Maccioni, R. B., & Seeds, N. W. (1986). Transglutaminase and neuronal differentiation. *Molecular and Cellular Biochemistry, 69*(2), 161-168.

Martin, A., Romito, G., Pepe, I., De Vivo, G., Merola, M. R., Limatola, A., et al. (2006). Transglutaminase-catalyzed reactions responsible for the pathogenesis of celiac disease and neurodegenerative diseases: From basic biochemistry to clinic. *Current Medicinal Chemistry, 13*(16), 1895-1902.

McGeer, P. L., Itagaki, S., & McGeer, E. G. (1988). Expression of the histocompatibility glycoprotein hla-dr in neurological disease. [Article]. *Acta Neuropathologica, 76*(6), 550-557.

Michaelsson, G., Ahs, S., Hammarstrom, I., Lundin, I. P., & Hagforsen, E. (2003). Gluten-free diet in psoriasis patients with antibodies to gliadin results in decreased expression of tissue transglutaminase and fewer ki67+ cells in the dermis. *Acta Dermato-Venereologica, 83*(6), 425-429.

Molberg, O., McAdam, S., Lundin, K. E., Kristiansen, C., Arentz-Hansen, H., Kett, K., et al. (2001). T cells from celiac disease lesions recognize gliadin epitopes deamidated in situ by endogenous tissue transglutaminase. *European Journal of Immunology, 31*(5), 1317-1323.

Nakaoka, H., Perez, D. M., Baek, K. J., Das, T., Husain, A., Misono, K., et al. (1994). Gh: A gtp-binding protein with transglutaminase activity and receptor signaling function. *Science, 264*(5165), 1593-1596.

Pinkas, D. M., Strop, P., Brunger, A. T., & Khosla, C. (2007). Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol, 5*(12), e327.

Porta, R., Esposito, C., Gentile, V., Mariniello, L., Peluso, G., & Metafora, S. (1990). Transglutaminase-catalyzed modifications of sv-iv, a major protein secreted from the rat seminal vesicle epithelium. *International Journal of Peptide and Protein Research, 35*(2), 117-122.

Pusztai, A., Grant, G., Spencer, R. J., Duguid, T. J., Brown, D. S., Ewen, S. W., et al. (1993). Kidney bean lectin-induced escherichia coli overgrowth in the small intestine is blocked by gna, a mannose-specific lectin. *Journal of Applied Bacteriology, 75*(4), 360-368.

Rakhimova, M., Esslinger, B., Schulze-Krebs, A., Hahn, E. G., Schuppan, D., & Dieterich, W. (2009). In vitro differentiation of human monocytes into dendritic cells by peptic-tryptic digest of gliadin is independent of genetic predisposition and the presence of celiac disease. [Article]. *Journal of Clinical Immunology, 29*(1), 29-37.

Reif, S., & Lerner, A. (2004). Tissue transglutaminase--the key player in celiac disease: A review. *Autoimmunity Review, 3*(1), 40-45.

Roth, E. B., Theander, E., Londos, E., Sandberg-Wollheim, M., Larsson, A., Sjoberg, K., et al. (2008). Pathogenesis of autoimmune diseases: Antibodies against transglutaminase, peptidylarginine deiminase and protein-bound citrulline in primary sjogren's syndrome, multiple sclerosis and alzheimer's disease. [Article]. *Scandinavian Journal of Immunology, 67*(6), 626-631.

Sarang, Z., Toth, B., Balajthy, Z., Koroskenyi, K., Garabuczi, E., Fesus, L., et al. (2009). Some lessons from the tissue transglutaminase knockout mouse. [Proceedings Paper]. *Amino Acids, 36*(4), 625-631.

Saudou, F., Finkbeiner, S., Devys, D., & Greenberg, M. E. (1998). Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell, 95*(1), 55-66.

Taurog, J. D., Richardson, J. A., Croft, J. T., Simmons, W. A., Zhou, M., Fernandez-Sueiro, J. L., et al. (1994). The germfree state prevents development of gut and joint inflammatory disease in hla-b27 transgenic rats. *Journal of Experimental Medicine, 180*(6), 2359-2364.

Trottier, Y., Lutz, Y., Stevanin, G., Imbert, G., Devys, D., Cancel, G., et al. (1995). Polyglutamine expansion as a pathological epitope in huntingtons-disease and 4 dominant cerebellar ataxias. [Article]. *Nature, 378*(6555), 403-406.

Tucholski, J., & Johnson, G. V. (2002). Tissue transglutaminase differentially modulates apoptosis in a stimuli-dependent manner. *J Neurochem, 81*(4), 780-791.

Visser, J., Rozing, J., Sapone, A., Lammers, K., & Fasano, A. (2009). Tight junctions, intestinal permeability, and autoimmunity celiac disease and type 1 diabetes paradigms. In M. Fromm & J. D. Schulzke (Eds.), *Molecular structure and function of the tight junction: From basic mechanisms to clinical manifestations* (Vol. 1165, pp. 195-205).

White, J. K., Auerbach, W., Duyao, M. P., Vonsattel, J. P., Gusella, J. F., Joyner, A. L., et al. (1997). Huntingtin is required for neurogenesis and is not impaired by the huntington's disease cag expansion. *Nature Genetics, 17*(4), 404-410.

Wilhelmus, M. M., van Dam, A. M., & Drukarch, B. (2008). Tissue transglutaminase: A novel pharmacological target in preventing toxic protein aggregation in neurodegenerative diseases. *European Journal of Pharmacology, 585*(2-3), 464-472.

Zainelli, G. M., Ross, C. A., Troncoso, J. C., & Muma, N. A. (2003). Transglutaminase cross-links in intranuclear inclusions in huntington disease. *Journal of Neuropathology & Experimental Neurology, 62*(1), 14-24.