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Delayed diagnoses in the spectrum of gluten-averse conditions

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DELAYED DIAGNOSES IN THE SPECTRUM OF GLUTEN-AVERSE CONDITIONS

By

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MASTER'S THESIS

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Crystal Maki
July 22, 2011

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A Thesis
Presented to
The Faculty of
Western Washington University

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ABSTRACT

Celiac Disease (CD), gluten allergy (GA), and non-celiac gluten sensitivity (NCGS) represent a highly varied disease grouping that affects individuals to varying degrees in response to the ingestion of certain cereal proteins (wheat, barley, rye, and sometimes oats). Generally, epidemiologic data on food allergy and intolerance is severely lacking; given current trends of under-diagnosis, prevalence of overt CD alone is estimated at 1-2% of European populations.

There is a large and growing body of scientific literature that ascribes the complexity of various gluten-sensitive symptomology to multiple developmental pathways. This complexity translates largely in to delayed clinical diagnosis by medical professionals. Furthermore, public awareness of gluten-averse reactions as a serious medical condition remains low; there likely exists a sizable amount of the population that displays symptoms but does not pursue a gluten free diet due to a lack of knowledge. These delayed diagnoses result in an extremely decreased quality of life for those affected and for those with undiagnosed CD, there exists an increased risk for the development of refractory celiac with fatal T-cell lymphoma. The goal here is two-fold: (1) to conduct an epidemiologic pilot survey geared toward the characterization of contemporary paths to diagnosis of a small sample of individuals with CD, NCGS and GA and (2) to use the results of this survey to make suggestions for decreasing time to diagnosis for this widespread contemporary health issue.

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INTRODUCTION

Celiac Disease (CD), Non-Celiac Gluten Sensitivity (NCGS), and gluten allergy (GA) represent a complex grouping of gluten-averse conditions that may be some of the most misunderstood, underdiagnosed, and easily treated conditions within the United States (Fasano et al 2003). Celiac disease (CD) is an immunogenetic disorder that affects multiple systems and displays a broad array of clinical presentations ranging from gastrointestinal symptoms only ("classic" CD) to gynecological, neurological, hematological, and many other non-gastrointestinal manifestations exclusively ("atypical") (Dickey 2009). Diagnosis of CD has traditionally rested upon histological examination of the proximal duodenum, wherein the presence of enteritis acts as the standard presence indicator. However, the nature of this multisystem ailment actually suggests the existence of a CD- gradient (Kagnoff 2007) for which allopathic diagnosis has been largely ineffective. For example, approximately 5% of persons with CD are mistakenly diagnosed with irritable bowel syndrome (Sanders et al 2001). Serological testing indicates that CD is prevalent among 1% of the world population. NCGS has only recently been acknowledged by medical research as its own entity separate from CD (Sapone et al 2011), and has yet to be universally acknowledged among physicians. Gluten allergy is more commonly diagnosed in children and prevalence estimates are less than 0.4% (Venter and Arshad 2011). Onset of all three of these conditions involves the ingestion of certain cereal proteins (glutens) and all are currently treated with a gluten-free diet (GFD).

Unfortunately, the complexity of these conditions has translated largely in to delayed clinical diagnosis by medical professionals. Furthermore, public awareness of gluten-averse reactions as a serious medical condition remains low; there likely exists a sizable amount of the population that displays symptoms but does not pursue a gluten free diet due to a lack of knowledge. These delayed diagnoses result in an extremely decreased quality of life for those affected and as delayed diagnosis is associated with an increased risk for the development of refractory conditions that do not respond to

treatment with a gluten free diet and greatly increased risk for T-cell lymphoma (3-6x). Previous research indicates that persons affected by CD in US often feel that their diagnosis was delayed (Green et al 2001) (n=1612, most diagnosed at ages 40-50). Current data shows that average delay for CD diagnosis in the United States is approximately 11 years (Fasano et al 2003). Such delays may reflect the lack of information and relatively low importance of food allergies from general practitioners and some internists (Gupta et al. 2008, 2009, 2010). As of yet, no one has characterized the delay in diagnosis for NCGS, but it is likely longer than that of CD given that it is only recently recognized. The goal here is two-fold: (1) to conduct an epidemiologic pilot survey geared toward the characterization of contemporary paths to diagnosis of a small sample of individuals with CD, NCGS and GA and (2) to use the results of this survey to make suggestions for decreasing time to diagnosis for this widespread contemporary health issue.

Provided below is: (1) An immunology primer to familiarize the reader with the terms and processes underlying immune system reactions; (2) an introduction to adverse food reactions including classification and relevant terminology; (3) An overview of glutens and their role in disease; (4) an overview of the gluten-averse conditions; and (5) and summary of the three gluten-averse conditions, with special attention to CD and NCGS.

IMMUNOLOGY PRIMER

The immune system is a widely distributed collection of tissue and cells that protect the body against various pathogens (Kindt et al 2008). It is highly adaptable and most importantly can distinguish self from non-self. It can be divided in to two major branches, **adaptive and innate immunity**. The innate response is the primary boundary against invasion from pathogens. It is non-specific in that its effectiveness does not rely on the precise recognition of pathogens. Instead, it seeks out generalized invaders, distinguished by common genetic patterns that are common to many pathogens. The **adaptive immune system** is a secondary line of defense. The key to its effectiveness is in the development of an immunological memory that allows highly specialized defenses to be mobilized in response to invaders, especially upon future encounters. Provided here is a basic overview of the adaptive immune system alongside an introduction to the Gell and Coombs Hypersensitivity Classification (1963). This physiological classification system can easily overlay the adverse food reaction classification and provide biological rationale for the differential symptomology of various allergic reactions.

THE ADAPTIVE IMMUNE SYSTEM

The adaptive process can be divided in to two major branches: **humoral and cell-mediated immune responses** (Figure 1) (Widmaier et al 2008). Both subdivisions involve a similar initial sequence of events that can be summarized as follows: the antigen encounters antigen-presenting cells that are programmed to grab hold of it and initiate an immune response. Populations of helper T-cells encounter the bound antigen and become activated such that they release **cytokines** (signals to other cells). It is important to note that the divisions of the immune system are somewhat arbitrary and there are many situations where these processes work together, often with overlapping function. **Antigens** are any substances that can bind specifically to an antibody or TCR (described shortly) (Kindt et al 2008).

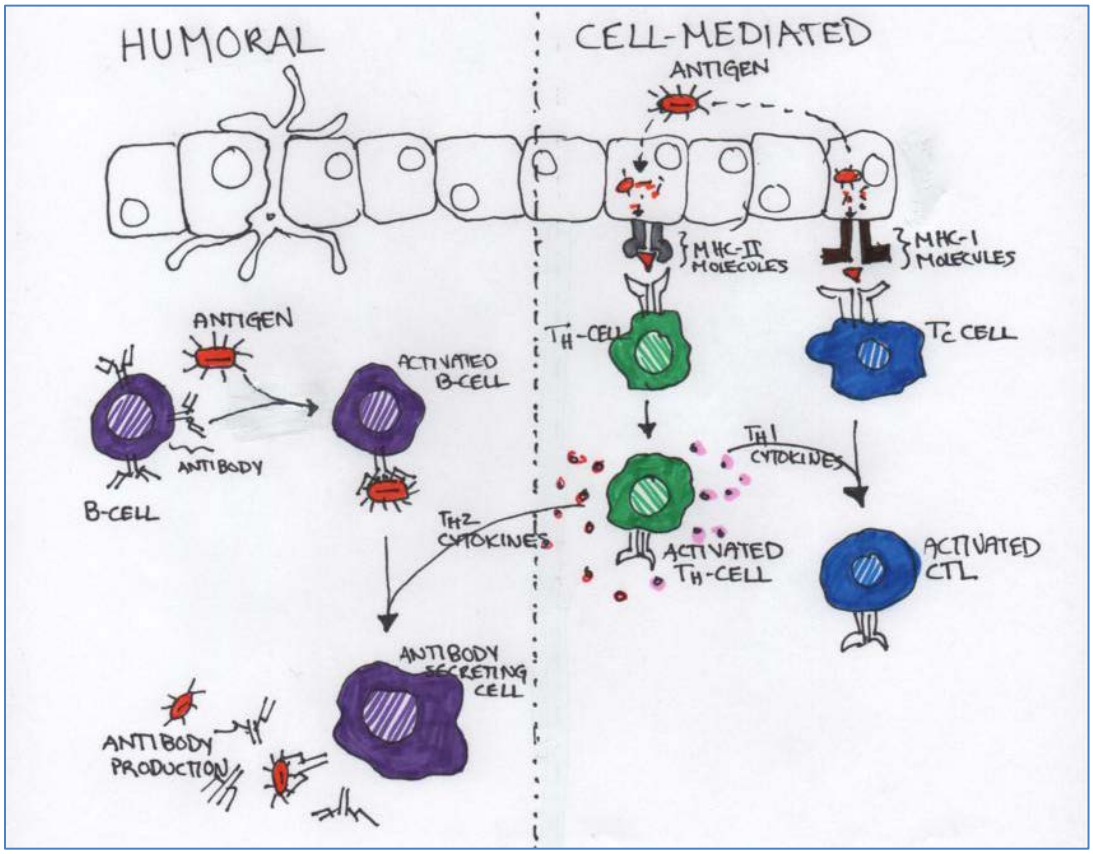

Figure 1. The Humoral and Cell-Mediated Immune response pathways. The Humoral response is focused on antibody production and is mediated by B-cells. Cell-mediated responses instead involve the production of activated cytotoxic lymphocytes (CTLs) and do not involve antibodies.

Immune-Cell Typology

There are many types of cells within the immune system (**leukocytes**): granulocytes, lymphocytes, mononuclear phagocytes, mast cells, and dendritic cells (Figure 2) (Kindt et al 2008). Each class of leukocyte can be differentiated by size, function, and approximate lifespan and contains further subclasses. The classification of immune cells is incredibly complex and only a few points and brief descriptions are covered here, taken largely from Kindt et al (2008) and Widmaier et al (2006).

(1) Granulocytes are leukocytes that exhibit a particular pattern of staining that shows a granular composition (Kindt et al 2008). The three subtypes are differentiated primarily by

the shape of their nuclei. **Neutrophils** and **Eosinophils** are both phagocytes that migrate from the blood stream in to damaged tissue. (Phagocytes are the immune cells that destroy harmful foreign items by ingesting and digesting them.) **Basophils** do not possess phagocytic function but instead play a role in cytokine (cell-signal) release in allergic responses. Their function approximates that of mast cells but occurs within the blood stream.

(2) **Mast cells** are a type of leukocyte that remains in their precursor form until they migrate from the blood in to skin, connective tissues, mucosal epithelium and the like. Upon migration they differentiate in to cells that carry a large amount of histamine and other cytokines important for the inflammatory/allergic response.

(3) **Monocytes** are a type of mononuclear phagocyte that is able to migrate from the blood stream into tissue and differentiate in to a **macrophage** with specialized function based on its target tissue (e.g. intestinal macrophages exist in the gut, osteoclasts in bone).

(4) **Dendritic cells (DCs)** are a class of leukocytes named for their many long fingerlike projections (similar to the dendrites of neuronal cells) (Satthaporn and Eremin 2001). Their main function is in antigen-presentation (Kindt et al 2008). Generally, they are present in areas of the body that have constant contact with the external world such as the skin and the gastrointestinal tract. Once they encounter an antigen they migrate to nearby lymph nodes and notify T- and B- cells.

(5) Lastly, **lymphocytes** are white blood cells of varying types produced in bone marrow that circulate through both the blood (20-40% of white count) and lymph systems (99% of lymph cell count). They can be divided in to three different types: **B-cells**, **T-cells** and **Natural Killer (NK) cells**. Special attention will be given to these cells in the next paragraphs due to their universal involvement in the initiatory stages of adaptive immune system responses.

All of these immune cells are derived ultimately from a single type of progenitor cell within the blood-hematopoietic stem cells (HSCs) (Kindt et al 2008). All leukocytes discussed here undergo at least two major type changes within their developmental trajectory. All 'intermediate forms' are only partially differentiated toward their end character and some are termed 'naïve'. For example, B-cells remain in a naïve form until their first encounter with an antigen at which point they mature in to memory B-cells (specific to the encountered antigen) able to produce antibodies. The timing and trajectory of HSC development and all intermediate forms are tightly controlled by numerous regulatory networks so that the appropriate type and amount of immune cells exist in the correct places at pertinent times in order to provide maximum protection against various pathogens.

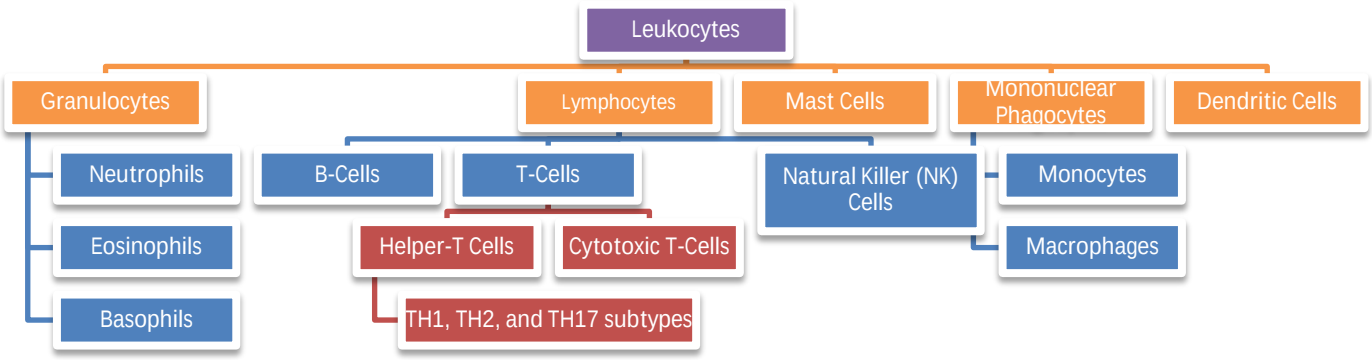

Figure 2: Major Leukocyte Subclasses.

B- and T- Cells

An understanding of B- and T-cell functionality is crucial for understanding the adaptive immune response: B-cells have antigen-presenting capability (Kindt et al 2008). The B-cell surface receptor responsible for binding the antigen is known as an **antibody (Ab) or immunoglobulin (Ig)**. These molecules (bound or free-roaming) are able to recognize and bind *free-roaming* antigens. Although specific to the antigen, there exist different classes (isotypes) of immunoglobulins: IgA, IgG, IgE, IgD, and IgM. These isotypes differ in location and function (see Table 1). Immunoglobulin A is of particular

importance in adverse food reaction because it is the primary antibody released in the gastrointestinal tract (Montiero et al 2010).

Antibody Isotype	Location	Functions (some)	% Total Serum	Number of Subtypes
IgA	Mucosal areas (e.g. digestive tract, bronchus, urogenital tract), breast milk, saliva, tears. Produced by B-cells that occupy sub-epithelial tissue (Montiero et al 2010)	Able to activate the complement system	10-15%, But comprises 70%+ of the Ig found in external secretions.	2
IgD	Antigen receptor on naïve B-cells.	Activate basophils and mast cells	<1%	1
IgE	Most IgE is bound to mast cells and basophils, some free-roaming	Bind allergens and signals for histamine release from mast cells and basophils (inflammation)	<<1%	1
IgM	Expressed on B-cell surfaces and also secreted in free roaming form	Involved in early stages of cell-mediated response (prior to IgG response), can activate complement pathway (most effective)	5-10%	1
IgG	Free roaming	Activate complement pathway, calls in macrophages and neutrophils, involved in antibody dependent cytotoxicity, Only antibody that can cross placental barrier.	approximately 80%	4

Table 1: The 5 Immunoglobulin isotype superfamilies.

T-cells require further development once they arise from bone marrow and proceed to the thymus gland in order to complete maturation ("T" is for thymus) (Kindt et al 2008). These T-cells do not have antibodies as the B-cells do. Instead the surface receptors responsible for binding antigens are called **T-cell receptors (TCR)**. T-cells are divided in to two types based on their particular TCRs: **Helper-T cells (T_H)** have CD4-type TCRs and **cytotoxic-T cells (T_C)** have CD8-type TCRs. T-cells (via their TCRs) only

recognize antigens that have already been bound by antigen-presenting cells (not free-roaming antigens). A third type of T-cell exists; T-regulatory cells contain CD4 type TCRs but have other surface proteins that make them functionally distinct. T_H cells come in further subtypes: **T_H1, T_H2,** and **T_H17**. T_H1 cells are involved in cell-mediated immunity and T_H2 cells are those that participate in the humoral response. T_H17 cells assist in inflammation. Activity of T_H1 cells inhibits the activity of T_H2 cells and vice versa, in order to keep the differential pathways from operating at the same time (and keep responses more specialized).

Cell-Signaling

The various cells of the immune system communicate with each other using specialized signals (Kindt et al 2008): **Cytokines** are a broad category of biochemical signals that include the interleukins (ILs), interferons (IFNs), and others (see Table 2 for some important examples). The large amount of signaling allows tight regulation of the various immune processes. Cytokines may act to promote or inhibit and many are pro-inflammatory (Dinarello 2000).

	Cytokine	Secreted by	Some Effects
Innate Immunity	IL-1	B cells, NK cells, macrophages	Promotes development (maturation) of B-cells, stimulates helper T-cells, activates NK cells, and promotes inflammation
	TNF-a	Macrophages	Inflammation, liver,
	IL-12	Macrophages, dendritic cells	Cytotoxic T-cell differentiation, causes NK cells to upregulate their IFN-γ and TNF-a production
	IL-6	Macrophages, endothelial cells	Antibody secretion, differentiation of various B, T, and plasma cells for immune response
	IFN-a	Macrophages	NK activation, increases MHC I expression
	IFN-b	Fibroblasts	NK activation, increases MHC I expression
	IL-15	Phagocytic cells	NK activation, suppresses cell apoptosis, T-cell regulatory roles
Adaptive Immunity	IL-2	T_H1	Promotes T-cell development
	IFN-γ	T_H1, T_C, NK cells	Promotes NK activation, T_H1 development, increases MHC expression (I and II)
	TNF-b	T_H1	Inhibits B-cell population expansion, inhibits

			macrophage activity
	IL-3	T_H1 and T_H2, NK cells	Promotes histamine release from mast cells
	IL-4	T_H2, mast cells, macrophages	Proliferation of T-cells, IgE synthesis from B cells, development and expansion of B-cell populations
	IL-5	T_H2, mast cells	Differentiation of B-cells and IgA synthesis, development of eosinophils
	IL-10	T_H2, macrophages	Stimulates macrophages to release cytokines, activates B cells, inhibits T_H1 cytokines, promotes T_H2 production
	IL-13	T_H2, B-cells	Stimulates B-cell populations and inhibits T_H1 and macrophage cytokine production
	TGF-b	T cells, macrophages	Inhibits B-cell populations and macrophages
	IL-18	Macrophages	Promotes NK activity, increases production of IFN-γ

Table 2: Common cytokines of the immune system, their source and general effects.

Antigen-Presentation

Nearly all cells in the body are able to present antigens to helper-T cells and elicit an immune response (Kindt et al 2008). T-cells only recognize antigens bound to very specific molecule types- those encoded by the massive gene complex known as the **Human Leukocyte Antigen (HLA).** Proteins coded by this complex can be subdivided in to two large groups: **Major Histocompatibility Complexes I and II (MHC I and II)**. MHC-I molecules are displayed by almost any nucleated cells in the body (non-immune) and can be divided in to three subtypes: A, B, and C (in humans). These molecules are composed of an a chain (coded by the MHC I) and a non-covalently associated b_2- macroglobulin chain. MHC-I molecules are used in signaling cytotoxic-T cells. MHC-II proteins are displayed by **antigen-presenting cells (APCs)** that include macrophages, dendritic cells, and B-cells. Some non-immune cells also express MHC-II molecules- fibroblasts, pancreatic beta cells, certain endothelial cells are some examples. All MHC class II proteins contain two chains (arms) (a and b) that physically assist in the binding and presentation of antigens. These molecules exist in different sub-types (DM, DQ, DR, and DP in humans) and are used to communicate with the helper-T cell populations.

Cell-mediated Response

The cell-mediated response protects the body against pathogens that have slipped past the innate and humoral defenses (present in the blood and lymph) and integrated themselves in to intracellular environments. Cell-mediated mechanisms can recognize and eliminate self-cells that have been infected and also cells that have undergone some sort of mutation (tumorous cells) (Figure 2, right column).

Two signals are needed to activate this response (Kindt et al 2008). A non-immune cell encounters an antigen and ingests it. The cell notifies the immune system that it has encountered an invader by presenting the antigen on its cell surface to local T_C cells using the MHC-I complex. APCs encounter the same antigen and present it via MHC-II molecules to populations of passing T_H1 cells. These helper T cells become activated release cytokines that induce cytotoxic T cells (that have encountered the MHC-I signals) to develop in to **cytotoxic T lymphocytes (CTLs)**. CTLs eliminate any 'altered' self-cells that have encountered the antigen. The development of CTLs specific for the pathogen takes time, which is why the cell-mediated response is 'delayed' onset and begins a few days after initial infection.

Cytokines produced by T_H1 cells include interferon-gamma (IFN-γ) and tumor necrosis factor beta (TNF-b) (Kindt et al 2008). Interferon-γ (IFN-γ) is responsible for the recruitment of macrophages ("big eaters") to the site of antigen encounter. Macrophages in turn signal for the up-regulation of MHC-II complex expression, further T_H1 production, and the inhibition of T_H2 activity through the secretion of IL-12 and IL-18. CTLs release their own set of signals which also include IFN-γ and TNF-b along with various cytotoxins that assist the CTL in destroying the target infected self-cell.

Humoral Response

The humoral response also involves T_H cells, but only to aid in B-cell maturation (not to activate CTLs) (Figure 2, left column) (Kindt et al 2008). This pathway is B-cell driven: B-cells encounter free-roaming antigens, bind, and display the intruder such that T_H2 cells can respond with cytokine production. The cytokines released in this process signal back to the B-cells and promote them (B-cells) to develop their antibody secreting abilities (B-cell maturation). The primary difference between the cell-mediated and humoral responses is that antibody production is only involved in the humoral response (not the cell-mediated response).

Antibody production cannot start without the appropriate signaling from T_H2 cells (Kindt et al 2008). Cytokines released by T_H2 cells include interleukins 4, 5, and 13. IL-13 and 4 are responsible for encouraging the production of IgE and some IgG antibodies. IL-4 is also the key player in the promotion of T_H2 development as well as T_H1 inhibition. IL-13 functions in the GI tract to increase mucus production and muscle contraction. IL-5 activates eosinophils to assist in elimination of any helminthes that have been bound by IgE. Cytokine signals used in the humoral and cell-mediated responses are almost entirely non-overlapping (Table 2).

In humoral immunity, the newly produced free floating antibodies bind the relevant antigens and mark them for removal from the body (Kindt et al 2008). To this end, merely binding antigens is not enough and additional signaling is required. There are a few major effects that can be mediated by signaling from antibodies: IgM (and most IgG) type antibodies can activate the **complement system**.

The complement is actually a portion of the innate system that can be brought in to play by the adaptive immune system, when helpful. It is comprised of a few types of proteins that circulate naturally in the blood as precursors to certain protein products. These proteins can do several things: (1) they can promote a process of antigen removal called opsonization wherein macrophages and neutrophils may be called in to phagocytose the harmful antigens. (2) Some of these complement

proteins have the ability to puncture membranes of various invaders and can thus assist in antigen removal by lysing (cutting open) the membranes of exogenous cells. (3) Some signals released by the immunoglobulins summon immune cells with cytotoxic functionality- **natural killer (NK) cells** especially. When NK cells arrive they are able to bind the free-floating antigen-specific antibody and use it to seek out the offending antigen for destruction. This process is called **antibody-dependent cell-mediated cytoxicity (ADCC)**. IgE has additional functionality that allows it to activate mast cells, eosinophils and basophils, all of which are important for the **inflammatory response**.

The humoral response differs depending on whether the antigen of interest has been previously encountered or not (Kindt et al 2008). If the antigen is new to the system, a 'primary' response is initiated. Antibodies produced by the first-time response are predominantly IgM and there is a focus on development of mature B-cells that are sensitive to the new antigen. In a secondary response however, preexisting populations of mature B-cells helps decrease response time greatly and their already considerable numbers are able to elicit a larger response. The predominant antibody produced by a secondary exposure is IgG. The development of antibodies against a particular antigen is termed **sensitization**. An individual is considered 'sensitized' to a particular substance if they have antibodies for it.

On a side note, NK cells are a nice example of the somewhat arbitrary nature of the divisions between adaptive and innate immunity and also between the two subtypes of adaptive immunity. NK cells are non-specific immune cells that can influence both the innate and adaptive responses. They can release cytokines (IFN-gamma) that promote the activity of macrophages (innate response). NK cytokines can also influence the development of Helper-T cells in to either T_H1 or T_H2 types. Lastly, the use of antibodies by NK cells in a process called Antibody-Dependent Cytotoxicity (ADCC) shows NK cells utilizing a product of the humoral response to carry out a function of cell-mediated immunity.

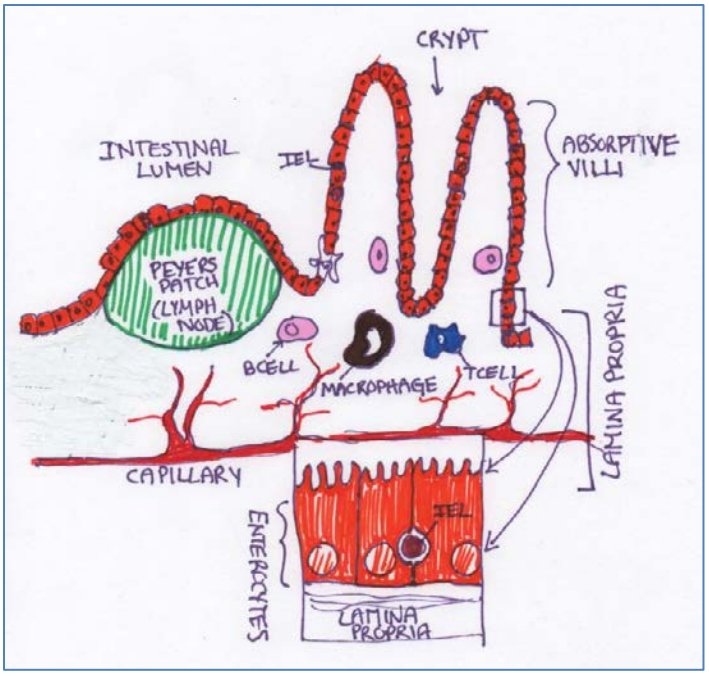

Figure 3. Gut-associated lymphoid tissue (GALT).

THE GASTROINTESTINAL TRACT AND IMMUNE FUNCTION

The lymph tissue of the gut bears the greatest burden in protection of the body due to its constant contact with the external environment. The antigenic load processed by **gut-associated lymph tissue (GALT)** within a single day far exceeds that handled by the systemic immune system within the human life time (Cochrane et al 2009). This gastrointestinal-associated lymph tissue (GALT) can be divided into three general compartments (Figure 3): Peyer's Patches, lamina propria, and intraepithelial spaces (Macdonald and Monteleone 2005). Peyer's Patches are the lymph nodes of the small intestine that contain local populations of macrophages, B-cells, T-cells, and dendritic cells. The lamina propria is the thin layer of connective tissue that lies beneath the epithelial layer that helps make up the mucosa of the gut. It also houses the capillaries that supply blood to the small intestine. (The mucosa, or mucosal layer, is comprised of the epithelial lining and the lamina propria.) The intraepithelial spaces are the small pockets of space between the epithelial cells that contain the intraepithelial lymphocytes. Overall,

the GI epithelium is responsible for nutrient absorption, barrier functionality, and the production of various substances for digestion and protection (Shimizu 2010).

Commensal Bacteria

Immune function is closely tied to the commensal bacteria of the gut throughout the human lifespan (Bjorksen 2004). At birth, the human gut is sterile. Within one week it is populated with various bacteria and within three months bacterial populations are generally settled into symbiosis with the gastrointestinal tract. The interactions involved are complex (Tanoue et al 2010). The commensals of the gut help to train the immune system during its most formative period (Cebra et al 1998; Hooper 2001). HLA-DQ genetics have been shown to have an effect on the progression of gut colonization in newborns (Sanchez et al 2011). A balance must be maintained throughout life through sustained barrier function and immunological tolerance (Kraehenbuhl and Corbett 2004; Sanz and De Palma 2009). Approximately 10^{14} bacteria (1 kg) live in an individual human small intestine alone, a number 10 times larger than the number of cells in the human body. At least 500 species of resident bacteria have been identified and this number is projected to be less than 50% of the species total (Guarner and Malagelada 2003). These collective bacterial colonies have been referred to as 'the forgotten' immune organ but are beginning to receive more recognition for their role in human health and disease (O'hara and Shanahan 2006). For example, Heijtz et al (2011) have discovered that gut microbiota play a role in brain development that may predispose for various behavior later in life (anxiety included). Depression is also known to develop from a combination of increased intestinal permeability and increased translocation of commensal bacteria into the submucosa (Maes et al 2008).

THE INFLAMMATORY RESPONSE

The inflammatory response is a series of events that is triggered when a pathogen makes it past the skin and mucous membranes (where innate immunity is most active) (Kindt et al 2008). The response may be immediate onset (acute) or chronic. The acute response is the body's primary method of repair for the initial stages of infection. Symptoms are easily identified: swelling, heat, pain, redness, and depending on the degree of inflammation, loss of function. Vasodilation allows increased blood flow to the site of infection. Vascular permeability is increased that allows fluid to accumulate in the area (edema). Once this has occurred, various leukocytes are prompted to migrate from the nearby bloodstream in to the infected area to assist in pathogen removal. These leukocytes also release cytokines that amplify the response until the pathogen has been effectively neutralized and tissue repair is complete.

HYPERSENSIVITY TYPOLOGY

Typically the immune response eliminates an antigen with minimal damage to the host's tissue. The mechanisms described above work together to bring about a targeted response that clears various viruses, bacteria, parasites, and any other foreign particles that are encountered. However, inappropriate immune system responses in some situations can cause injury or death (Kindt et al 2008).

These inappropriate and damaging immune responses are termed **hypersensitivities**, of which there are 4 main types (Gell and Coombs 1963) (Table 3). For ease of comparison, the three conditions relevant to this study are printed in bold-face type in the table. Of course, there are some disorders that could fall under multiple categories or have not yet been characterized well enough to classify. Remember, here the term 'hypersensitivity' is used more broadly than as defined by the NIAID (Boyce et al 2010). This is because it is a *classic* categorization of allergy that long predates the recent NIAID report; it is used universally in immunology and medical practice.

An **allergen** is commonly described as anything that can cause an allergic reaction (Kindt et al 2008). Some have used the term more specifically to refer to any nonparasitic antigen capable of bringing about a type 1 (anaphylactic) reaction. The NIAID describes food allergens as any component of food that can induce an immune response (Boyce et al 2010). Here the latter definition will be used: any antigen that elicits an adverse immune response is called an allergen.

Types 1, 2, and 3 hypersensitivities occur within the humoral response and involve an 'immediate' reaction of a sensitized immune system to the encounter of the allergen. These reactions manifest within minutes to hours of exposure (Kindt et al 2008) and are distinguished by their predominant mediators and corresponding effector molecules (discussed shortly). Type IV hypersensitivity on the other hand occurs during cell-mediated immunity and is delayed onset (days)

Within each of these four categories, one can subdivide by source of the antigen. In some cases the antigen is from outside the body (exogenous) and an **allergy** results; in other cases the body's immune system develops a memory for some part of the self and an **autoimmune disorder** develops. All hypersensitivities denote 'allergies' because they involve an inappropriate immune response of some kind, whether the antigen is exogenous or not. To describe an autoimmune disorder as an allergy is technically correct given the definitions provided here. However, the differences in pathology underlying these distinctive varieties of allergy are considerable and the distinction between general allergy and autoimmune disorders should not be taken lightly. Here, 'allergy' and 'autoimmune disorder' are used to distinguish the source of the antigen.

Gell and Coombs (1963)	Proximal Cause	Mediators	Source of Antigen	Some Example Conditions
Type I: IgE-Mediated/ Anaphylactic	Cross-linking of IgE on mast cells causes degranulation and release of vasoactive mediators	IgE	Foreign	Atopic dermatitis, **Classic food allergy (milk, egg, peanut, wheat …)**
			Autoimmune	None
Type II: Antibody Dependent Cytotoxicity	Antibodies are directed against cell surface antigens which cause destruction through activation of the complement system or ADCC	IgM or IgG	Foreign	Pernicious anemia
			Autoimmune (cytotoxic)	Rheumatic fever, Autoimmune hemolytic anemia, Bullous pemphigoid
			Autoimmune	Graves' disease, Myasthenia Gravis
Type III: Immune Complex Mediated	Antigen-antibody complexes bring about complement activation and large scale inflammatory response (through influx of neutrophils)	IgG	Foreign	Reactive arthritis, Rheumatoid arthritis, Serum sickness, Arthus reaction
			Autoimmune	Systemic lupus erythmatosus, Sub-acute bacterial endocarditis
Type IV: Delayed onset hypersensitivity	Sensitized helper-t cells release cytokines that activate cytotoxic cells or macrophages that target 'infected' cells for destruction.	T-cells, CTLs especially	Foreign	Allergic contact-dermatitis, **Non-Celiac Gluten Sensitivity**
			Autoimmune	Type-1 diabetes, Hashimoto's Thyroiditis, Guillian-Barre Syndrome, Multiple Sclerosis
Unclassified/Mixed	Unknown/mixed	Unknown/ mixed	Foreign	Transplant rejection, Latex allergy (1 and 4)
			Autoimmune	Sjogren's syndrome, Autoimmune Hepatitis, **Celiac Disease (Types II and IV)**

Table 3: Overview of Hypersensitivity Disorders. Note: Classification of NCGS is still in the works. The mechanisms that underlie this condition are not yet fully elucidated (see Non-Celiac Gluten Sensitivity).

TYPE I HYPERSENSITIVITY

Type I hypersensitivity is the most studied of the four types- likely because it involves rapid onset symptoms and tends to be a concern more often in childhood (Sampson 1999). In this process, IgE antibodies produced by the humoral response bind tightly to mast cells and basophils (a process called sensitization, mentioned earlier). Upon second exposure to the allergen, an environment is created wherein bound antigens encourage the cross-linking of the antigen-specific IgE of various mast cells. (Cross-linking is where an antigen binds two different antibodies simultaneously on the same mast cell). This cross-linking in turn triggers the mast cells to degranulate[1] (disintegrate) and release a large amount of chemical signals in to their immediate environment (see Table 4). Some of these signals are released directly from the mast cells (primary action) and some are a downstream effect of the primary signals (secondary actions). These signals include vasoactive mediators (e.g. histamine) that are able to cause smooth muscle contraction, vasodilation, and increase vascular permeability. In other words, IgE invokes an inflammatory response. Cytokines are released that may promote systemic anaphylaxis and further increase the production of IgE. Degranulation cytokines include: IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF, TNF-a, and others. Some of these cytokines function to bring in other specialized immune cells including eosinophils and neutrophils (chemotactic function).

[1] Crosslinking is one of 5 recognized triggers of mast cell degranulation (Kindt et al 2008).

Action	Mediator	Effect
Primary	Histamine, heparin	Increase vascular permeability, smooth muscle contraction
Primary	Proteases	Increase mucus production from bronchial tubes, degrade blood vessel basement membrane
Secondary	Platelet activating factor	Platelet aggregation and degranulation
Secondary	Leukotrienes	Increase vascular permeability; pulmonary muscle contraction, broncho-constriction
Secondary	Prostaglandins	Vasodilation, platelet aggregation, pulmonary muscle contraction
Secondary	Bradykinins	Increase vascular permeability, smooth muscle contraction
Secondary	Various cytokines	Increase IgE production (IL-4 and IL-13), systemic anaphylaxis (IL-1 and TNF-a)

TABLE 4. Mediators released by the degranulation of mast cells in a type 1 reaction (primary) and those formed secondary to the release of the primary mediators.

Normally, the IgE response is used to fight parasitic infections, particularly helminthes (Kindt et al 2008). However, some genetically predisposed persons have a condition that allows them to develop these reactions toward various natural environmental components (Boyce et al 2010). This condition is called **atopy or atopic syndrome.** These individuals often have 10x the normal amount of naturally circulating IgE (Kindt et al 2008:374). Common symptoms of localized type 1 hypersensitivity reactions include atopic dermatitis (a type of eczema), urticaria (hives), asthmatic attacks, allergic rhinitis, vomiting and diarrhea (Boyce et al 2010). All of these may occur in response to ingested food allergens. **Cross-reactivity** is also a concern in the development of type 1 hypersensitivity (Breiteneder and Mills 2006).

TYPE II HYPERSENSITIVITY

In Type II hypersensitivity, antibodies interact with membrane bound 'antigens' and either (1) activate the complement system or (2) initiate antibody dependent cell-mediated cytotoxicity (ADCC). The error occurs when there is a failure of tolerance to a naturally occurring element in the body or when a true antigen happens to resemble a naturally occurring cell surface molecule. In this second case antibodies are made against the antigen but they are also able to cross react with the naturally occurring element

and an inappropriate response is elicited. Macrophages recognize and bind these antigens and present them to B-cells so that antibody production can begin. In both cases, any cell to display the offending antigen is marked for destruction. Common examples of this category include autoimmune hemolytic anemia, Graves' Disease, and pernicious anemia.

TYPE III HYPERSENSIVITITY

In Type III hypersensitivity, antigen-antibody complexes (IgM or IgG) cluster in unnaturally large amounts in various tissues (Kindt et al 2008). Normally these complexes are marked for destruction however the buildup of these clusters can activate the complement pathway such that a massive inflammatory response is brought about and a large amount of neutrophils are brought in to assist in the phagocytosis of the perceived offender. Neutrophils further amplify the inflammatory signals by releasing their own cytokines. Most commonly this clustering occurs in blood vessel walls, joints, parts of the kidney and the cerebrospinal fluid compartments of the brain (choroid plexus). Type III reactions can be localized or generalized depending on the location of the complex deposits. Disease examples include lupus erythematous and rheumatoid arthritis.

TYPE IV HYPERSENSIVITITY

Type IV hypersensitivities occur during the cell-mediated immune response and are often referred to as Delayed-Type Hypersensitivity (DTH) (Kindt et al 2008). The cell-mediated immune process proceeds normally (as described above) but is in reaction to an inappropriate stimulus. Key components to DTH include (of course) the time delay until onset, as well as the recruitment of macrophages (instead of neutrophils). Cell-mediated immunity is an important defense against intracellular antigens; various bacteria, fungi, parasites, and viruses travel via intracellular pathways. Type I diabetes and allergic contact dermatitis are examples of this category.

ADVERSE FOOD REACTIONS

Celiac Disease, wheat allergy, and non-celiac gluten sensitivity are all clinically different conditions with distinctive pathophysiology. Commonalities exist between these conditions: all are triggered by the ingestion of gluten, and a gluten free diet (GFD) is the only current treatment in all cases. These common features, coupled with a lack of reliable diagnostic criteria have led to extensive confusion among both medical professionals and the general public regarding these various conditions (Boyce et al 2010). Clinical presentation of these conditions *can be* similar, but this is not the rule. The distinction between conditions remains important primarily because differential symptomology can aid health professionals (and individuals) in diagnosis. A better understanding of these conditions is therefore preceded by a working understanding of adverse food reaction classification (allergy, intolerance and the like). **Adverse food reactions** include any clinically abnormal reaction to a given food (Anderson 1991). Provided here is an overview of adverse food reactions with special attention to gluten-averse conditions, as well as definitions and clarifications on relevant terminology.

Below is a table of the three known classifications of gluten-averse conditions (Table 5) - Celiac Disease (CD), Gluten Allergy (GA), and Non-Celiac Gluten Sensitivity (NCGS). These are the names that will be used throughout this project, and were chosen because they are in line with the definitions of allergy and intolerance as put forth by the NIAID (Boyce et al 2010). Synonyms are listed to the far right, some of which are to be avoided for reasons listed below. Here we will explore two separate but largely overlapping classification systems under which these conditions may be sorted- one put forth by the NIAID to classify adverse food reactions (Boyce et al 2010), and also the classic typology for adverse immune system reactions described in the hypersensitivity section (Gell and Coombs 1963).

Name	Adverse Food Reaction Classification	Hypersensitivity Type	Onset of Symptoms	Other Names (*some improper*)
Celiac Disease (CD)	Non-IgE Mediated Humoral Immune Response	Auto-Immune Disorder/ Mixed Classification (Types II and IV)	Variable (some immediate, some delayed symptoms)	Celiac Sprue, Sprue, non-tropical Sprue, Gluten sensitive enteropathy (GSE), *Gluten intolerance*
Gluten Allergy (GA)	IgE-Mediated Humoral Immune Response	"Classic" Allergy/ Type I	Immediate Onset (within minutes to hours)	Wheat Allergy, *Gluten intolerance*
Non- Celiac Gluten Sensitivity (NCGS)	Cell-Mediated Immune Response	Allergy/ Type IV	Delayed Onset (within hours to days)	Gluten sensitivity* *Gluten Intolerance*
Gluten Intolerance	Intolerance	N/A	Condition is undocumented in medical literature	None

Table 5. Gluten-averse conditions, including differences in classification and alternative terminology. Terms shown in italics are other names that are used *improperly* to refer to the condition listed. Non-italicized terms in the 'other names' column are true synonyms. *Gluten sensitivity has been used by some to refer to any and all gluten-averse reactions.

CLASSIFICATION

Adverse food reactions can be broadly divided into those that involve the immune system and those that do not (see Figure 4). This primary division is illustrative of the differences between **food allergy** and **food intolerance** (Anderson et al 1991; Ortolani et al 2006). In food allergy, the adaptive immune system responds reproducibly whenever an individual is exposed to the food allergen (Boyce et al 2010; Sicherer and Sampson 2010). In contrast, the adverse reaction generated from food *intolerance* occurs for non-immune related reasons (Anderson 1986). For example, a person may be lactose intolerant because their body does not produce sufficient amounts of lactase to aid in digestion. There exists a third large category (not shown) that includes secondary sensitivities where an individual may have an adverse reaction to a given food due to the effects of some other condition (e.g. drug-induced

sensitivities) (Taylor 2001). Wheat allergy and celiac disease both involve the immune system and are classified here under the allergy category. Note: CD is an autoimmune disorder, the pathophysiology of which relates directly to the ingestion of gluten (food). However, it is not a 'food allergy' in the same sense as wheat allergy. The reaction is food related, but the inappropriate immune response involved is against a naturally existing (endogenous) protein in the body (see Celiac Disease Pathophysiology section).

The entire immune-mediated category of adverse food reaction pertains to responses from various parts of the adaptive immune system. Within immune-mediated adverse food reactions there are those that are directed by the humoral immune response (immediate) and those that involve cell-mediated immunity (delayed). Immediate responses can be further divided based on the primary antibody class involved. **"True" allergies** involve an IgE-mediated response, sensitization and reaction upon future encounters to the allergen (Venter and Arshad 2011). However, sensitization, or the presence of IgE specific for the food allergen, does not completely predict a negative reaction upon second exposure (Boyce et al 2010). A positive correlation exists between level of IgE and chances of negative reaction and some researchers have argued for a predictive cutoff point (Boyce et al 2011). Thus far, no cutoff has been established. The basic physiology of allergy and the responses mentioned here will be covered in the upcoming section on adaptive immune system. In any case, allergy as a classification still encompasses any immune-mediated adverse reaction to food.

Adverse food reaction classification can be overlaid with the Gell and Coombs (1963) classification scheme for hypersensitivity to help further illustrate the underlying pathophysiology of immune-mediated reactions (refer again to Figure 4). IgE-mediated reactions are entirely synonymous with a type I hypersensitivity. Both Type II and type III hypersensitivities are non-IgE mediated. All cell-mediated (delayed onset) adverse food reactions fall under the type IV hypersensitivity category.

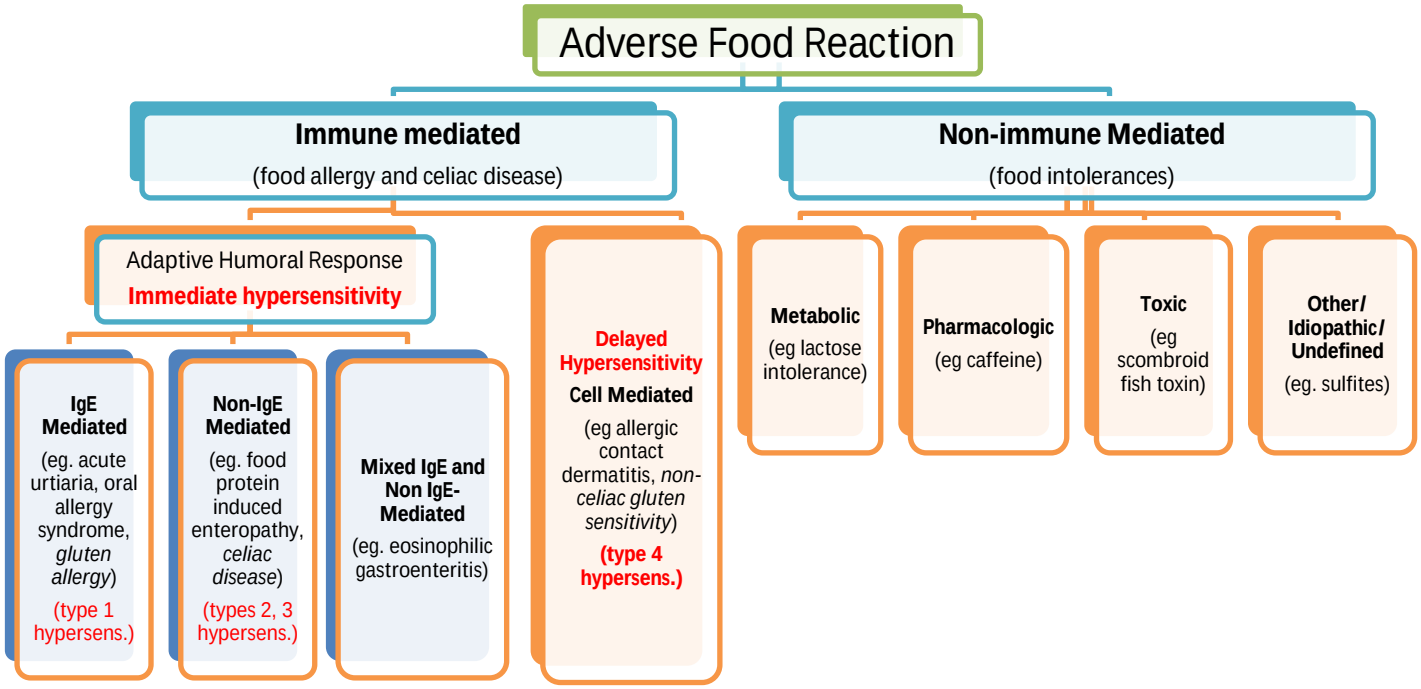

Figure 4. Classification of Adverse Reactions to Food based on Pathophysiology with corresponding Gell and Coombs Hypersensitivity Classification (1963) (adapted *from* Boyce et al 2010).

TERMINOLOGY: DEFINITIONS AND CLARIFICATIONS

The various gluten-averse conditions have been described many different ways (see Table 5) and current terminology-usage is confusing for all. The creation and use of standardized terminology is paramount to clearing up the confusion over these three gluten-averse conditions. Provided here are some definitions and clarifications aimed at standardizing the terminology for gluten-averse conditions:

(1) The term **hypersensitivity** is sometimes used synonymously with allergy, and sometimes as a broader term to include food intolerances. The National Institute of Allergy and Infectious Disease (NIAID) Guidelines Report uses the term only in reference to IgE-mediated adverse reactions that take place within the gastrointestinal tract. Here, we will use the term more broadly to refer to any immune-mediated adverse reaction, as is consistent with the immunological terminology of the Gell and Coombs Classification System (1963), which long

predates the recent NIAID report. Food intolerances can be referred to as non-allergic hypersensitivities (Johansson et al 2001).

(2) **Tolerance** has two different definitions, provided by two different spheres of authority. According to the NIAID, tolerance is a condition where a person no longer exhibits symptoms of food allergy- either through outgrowing the allergy naturally or through therapy. If a person has never had an allergy to a particular allergen, they are *not* said to have 'tolerance' for the allergen. However, in general immunologic terms, tolerance is also a state of protection of the self against its own immune system (Kindt et al 2008). Here we will use the second and more general definition of tolerance. In either case, intolerance is not an appropriate term to use as an opposite for tolerance. Intolerance is still only defined as a non-immune mediated adverse food reaction. The common root is not considered meaningful for these two terms. In the case of our second tolerance definition, the reverse situation is referred to as 'failed tolerance'.

(3) The terms '**gluten intolerance**' and '**gluten sensitivity**' are both used in a wide variety of circumstances, contributing greatly to the confusion about the different types of gluten-averse reactions. Some people use these terms as catch-all classifications for *any* adverse gluten reaction (allergy and celiac disease). Some have used these terms as labels to refer more specifically to the delayed reaction to wheat, characteristic of the cell-mediated immune response (e.g. Food Intol.org 2011). In any case, gluten 'intolerance' is not an 'intolerance' as officially classified by the NIAID; *it is a misnomer*. A non-immune mediated adverse reaction to glutens has not been documented in medical literature. The classification is correct only as far as differentiating it from a 'true' allergy which induces an immediate response (within hours). However, the response is immune-related nonetheless and still falls under the category of allergy. Here, the term Non-Celiac Gluten Sensitivity (NCGS) is chosen to refer to this form of delayed-- onset adverse gluten reaction.

(4) Gluten Intolerance may exist as a condition (non-immune adverse reaction) although it has yet to be described in medical literature. Currently, 'gluten intolerance' as described in published literature involves an immune component and is thusly not an 'intolerance' but an allergy. For example, "Gluten intolerance is a common, immunologically mediated disorder with a widely variable clinical presentation that affects genetically predisposed subjects" (Bardella et al 2005:15).

It seems reasonable that a term be chosen to represent the spectrum of gluten-averse conditions. Gluten sensitivity is the best candidate of those in current use. However, there are some downsides to its adoption as the catchall term. (1) Non-Celiac Gluten Sensitivity would sound as if it is defined in opposition to Celiac Gluten Sensitivity, implying that NCGS would also include wheat allergy. (2) Gluten sensitivity as a term is already used by some to describe the third condition (NCGS) and those using the term would need to re-clarify their descriptions as NCGS. It could be argued that due to its use in a variety of definitions that it should be abandoned on the whole and an entirely new term should be created. No matter the adopted broad term, a large scale adjustment will need to take place regarding usage and it will need to be recognized that publications prior to the approximate change must be read carefully. Here, a new term '**gluten-averse condition**' is created to be used as the broad scale term that includes CD, GA, and NCGS.

Inconsistencies in terminology are at least partly reflective of the inconsistencies in adverse food reactions more generally (Gupta et al 2007). Misuse continues despite the implementation of standardized terminology on adverse food reaction from the World Allergy Organization beginning in 2001, many definitions of which predate the 1980s (Johansson et al 2001; Johansson et al 2004) (*see* Zopf et al 2009 as an example). Publicly available information is wrought with inaccuracies, which undoubtedly contributes to the spread of misinformation, and in turn a large amount of missed

diagnoses for people attempting to self-diagnose. A quick internet search for 'celiac disease', 'wheat allergy' and 'intolerance' turned up the following examples of misuse (see Table 6).

Source	Improper Terminology Use	Comment
National Institute for Allergy and Infectious Disease[2]	"Gluten intolerance: Gluten intolerance is associated with celiac disease, also called gluten-sensitive enteropathy. This disease develops when the immune system responds abnormally to gluten. This abnormal response does not involve IgE antibody and is not considered a food allergy."	The NIAID, whose standards are used in describing allergy and intolerance, uses the term intolerance to describe an immune-mediated reaction (allergy)
Food Intol.org[3]	"What is the difference between Wheat Allergy and Wheat Intolerance? For clarity they are NOT the same thing: *Wheat Allergy is a severe sudden onset allergic reaction to a certain protein component of wheat. That is, it's an auto-immune response* of the body. … It can cause life-threatening responses in allergic people….HOWEVER, most people who speak of wheat allergy are really referring to Wheat intolerance caused by Gluten …*Wheat Intolerance is when you have difficulty digesting wheat, which may seem less important. It is a slower onset but certainly involves the immune system…"*	FoodIntol.org mistakenly described wheat allergy as an autoimmune condition. (Celiac Disease is the autoimmune condition.) They also use the term 'gluten intolerance' to refer to an immune-mediated reaction.
American Association of Retired Persons (AARP)[4]	"Celiac disease is an inherited, autoimmune disease in which the lining of the small intestine is damaged from eating gluten and other proteins found in wheat, barley, rye, and possibly oats. Sprue; Nontropical sprue; Gluten intolerance; Gluten-sensitive enteropathy"	The AARP lists gluten intolerance as a synonym for Celiac Disease.
ExitAllergy.com[5]	"What Is Gluten Allergy? The digestive disorder *coeliac disease (gluten allergy)* scars the lining of the small intestine preventing the absorption of nutrients. Gluten allergy symptoms manifest as gastrointestinal distress of all forms."	Exit Allergy.com uses Celiac Disease and Gluten allergy as synonyms.
MayoClinic.com[6]	*"Common food intolerance conditions that are often mistaken for food allergies include: ….Celiac disease*. This	The Mayo Clinic incorrectly places Celiac Disease under

[2] http://www.niaid.nih.gov/TOPICS/FOODALLERGY/UNDERSTANDING/Pages/foodIntolerance.aspx
[3] http://www.foodintol.com/wheat.asp
[4] http://healthtools.aarp.org/adamcontent/celiac-disease-sprue?CMP=KNC-360I-GOOGLE-HEA&HBX_PK=gluten_intolerance&utm_source=Google&utm_medium=cpc&utm_term=gluten%2Bintolerance&utm_campaign=G_Diseases%2Band%2BConditions&360cid=SI_148893851_6495451981_1
[5] http://www.exitallergy.com/allergy-articles/diagnosing-gluten-allergy.php
[6] http://www.mayoclinic.com/health/food-allergy/AN01109

	chronic digestive condition is triggered by eating gluten, a protein found in bread, pasta, cookies, and many other foods containing wheat, barley or rye. Signs and symptoms of celiac disease include diarrhea, abdominal pain and bloating. While celiac disease involves an immune system response, it's a more complex food reaction than a food allergy."	the intolerance category.

Table 6: Examples of wide-spread misuse of terminology for the gluten-averse conditions.

These points on terminology illustrate nicely the considerable amount of confusion on part of both the public and health professionals when it comes to describing gluten-averse responses and adverse food reaction in general. In particular, misuse of the term 'intolerance' is widespread even within peer-reviewed research publications, sources that should be considered both reliable and authoritative. For example, Granzotto et al (2009) describe Celiac Disease as "characterized by intolerance to gluten" (p 1513). Freeman et al (2011) and many others can be quoted identically.

Summarily, standardized terminology has yet to be mass-applied and one must keep this in mind when reading publications that involve wheat allergy, celiac disease, and adverse gluten-reactions, as well as allergy and intolerance, in general. All attempts have been made here to utilize the official terminology set forth by the NIAID in their 2010 publication (Boyce et al 2010).

FOOD ALLERGY OVERVIEW

The importance of adverse food reaction as a medical condition can be best illustrated by a social contextualization of impact. An epidemiologic overview of food allergy is provided prior to discussing the three gluten-averse conditions. Celiac Disease will be discussed in further detail within the next section.

Although food allergy represents a significant health concern, especially among children, basic epidemiologic information is lacking (Lack 2008). Over 170 foods have been documented to cause IgE-

mediated allergic reactions (Boyce et al 2010). The most common ones ("Big Eight") include: wheat, shellfish, eggs, fish, peanuts, milk, tree nuts, and soybeans (Taylor 2001) and account for over 90% of all adverse food reactions (Branum et al 2008). Food allergy occurs almost exclusively in response to ingested proteins (Radauer et al 2008) and as mentioned above can cause either immediate immune response (minutes to hours) or delayed hypersensitive reaction (24 hours or later) (Taylor 2001). Adverse reactions range from mild depression and gastrointestinal discomfort to anaphylactic shock and death (Boyce et al 2011). Despite the clear potential to benefit, there continues to be an absence of universal diagnostic criteria (Venter and Arshad 2011).

Estimates of incidence and prevalence are highly varied and any synthesis and comparison of such estimates is currently impossible. A recent meta-analysis by Rona et al (2007) summarizes the findings of 51 studies- food allergy (self-reported) varies between 3-35% of the total population (regardless of location, but largely dominated by Western groups). Clinically confirmed food allergy by (oral food challenge) estimates between 1-10.8% prevalence, based on 6 studies. Conservative estimates currently indicate that 6-8% of children and 4% adults have food allergies and/or intolerances (Bangash and Bahna, 2005; Burks and Ballmer-Weber, 2006; Sicherer and Sampson, 2006) although this cannot be verified. Public knowledge on adverse food reactions is dominated by a tendency for people to classify any adverse reaction as food allergy and tends to result in an over-reporting of allergy incidence: self-diagnosed food allergy may be as much as 10 times higher than the true value attainable by medical testing (Venter and Arshad 2011). Thus, a range of possibility exists for the true prevalence of food allergy and the measures of central tendency remain elusive.

Provided here is a summary table of food allergy prevalence estimates per allergen (Table 7). Note that these range estimates are derived from studies of varying methodology and it is not possible to report fairly anything more than a range. Additionally, the division between adults and children (age 18) is not necessarily biologically meaningful for the purposes of allergy development. Countries

represented in these estimates are predominantly Western groups- outside of the Western world estimates of allergy prevalence are even more sparse.

ALLERGEN	ALLERGY PREVALENCE				
	Children	**Age**	**Adults**	**Age**	**Countries Included**
Milk	0.2-2.5%	under 18	0.1-1.1%	18-60	France, UK, US, Denmark, China, Israel
Egg	0.2-5.0%	under 18	0.2-1.3%	18-60	France, UK, US, Denmark, China, Australia
Wheat	0-0.4%	under 15	0-1.4%	18-60	US, Australia
Fish	0-0.2%	under 18	0.1-0.6%	18-60	US, Canada, UK, Denmark, Germany, Australia
Shellfish	0-1.8%	under 18	0-2.5%	18-60	US, Canada, UK, Denmark, Germany, France
Peanut			0.06-5.9%	1 - 85.	US, Canada, France, Germany, Sweden, UK, Australia, Israel, Singapore, Philippines,
Tree nut			0.03-8.5%	1-65.	US, Canada, France, Germany, Sweden, UK, Australia, Israel, Singapore, Philippines,

Table 7. Prevalence ranges per food allergen, per age group, and associated countries included in estimate. Prevalence here is based on sensitization and reaction. (Info from Venter and Arshad 2011).

Traditionally, food allergy has been a concern mostly in early childhood- a large amount of effort has focused on childhood allergy and its potential 'remission'. One study shows annual incidence of clinically diagnosed food allergy decreasing from 4.7% to 1.2% from the ages 1-2 through ages 5.6 years of age (Schnabel et al 2010). Children affected by food allergy are more likely (2x-4x) to suffer from related conditions including asthma, eczema, and other respiratory allergy (Branum et al 2008). It is generally accepted that the neonatal period developmentally important in terms of tolerance (Brandtzaeg 2002), a time that corresponds to the development of a healthy commensal flora in the gut begins shortly after birth (Indrio et al 2011).

There is some evidence that food allergy prevalence varies by geographic region and population, although this cannot be confirmed without consistent diagnostic criteria (Freeman 2010). Any variation that does exist cross-culturally is likely the result of differential human genetic and epigenetic backgrounds (Tjon et al 2010) and also a function of cultural patterns for the breeding, storage, use and preparation of preferred foods (Shek and Lee 2006).

Unfortunately, delayed onset (cell-mediated) allergic reactions have not received as much attention as their immediate onset counterparts- which are much easier to diagnose (Jyonouchi 2008). Non-IgE mediated reactions are typically not life-threatening: Generally, delayed reactions are often more mild, appear days post-contact/ingestion, and are typically diagnosed later in life (among adults). "True", or anaphylactic, allergic reactions have received the bulk of epidemiologic and medical research thus far and unfortunately there is very little to no information on which foods might be most commonly responsible for cell-mediated (delayed) allergic response (Venter and Arshad 2011).

Increasing Prevalence of Food Allergy

Current research points almost universally to an increase in food allergy prevalence (all allergens) within recent decades, even after controlling for diagnostic improvement (Bjorksten 2004; Cochrane et al 2009). For example, the U.S. Department of Health and Human Services reports that between 2004 and 2006, hospitalizations due to food allergy for individuals under the age of 18 increased from approximately 2,000 to 10,000 (Branum et al 2008). Food allergy rose by 18% among children under age 18 from 1997 to 2007. In 2007 alone 3 million food allergy diagnoses were made among children (under age 18). This information is taken from the National Hospital Discharge Survey (NHDS); any food allergies diagnosed by parents in the home in which children were not hospitalized are not included in this estimate.

The apparent universal rise in allergy, coupled with increased knowledge of immune development and regulation, has skewed much research from questions of individual pathogenesis toward why lack of affect among all individuals (Bjorksten 2004). As of 2011, the hygiene hypothesis is the forerunner for best explanation of increasing allergy rates (See Hygiene Hypothesis). Less popular theories include the Dietary Fat Hypothesis, Antioxidant Hypothesis and the Vitamin D Hypotheses (both increased and decreased) (see Lack et al 2008 for a review).

HYGIENE HYPOTHESIS

The hygiene hypothesis was proposed by Strachan (1987) to account for the apparent increase in allergy over the past century. He suggests that improved hygiene and interest in cleanliness have decreased the occurrence of infection and microbial insult in children and thereby increased the incidence of atopic diseases. This claim originated largely from the observation that the children of farmers have a much lower incidence of allergy, and those of academic parents exhibit allergy more often (Ring et al 2004). Indeed there is substantial evidence to support the theory that a lack of immunological training early in life may predispose to allergy (Kalliomaki and Isolauri 2002). Immunologically it is claimed that early exposure to various pathogens (infection, parasites, vaccinations) skews the T_H1/T_H2 responses to favor T_H1 and thereby protect against IgE-mediated allergy development by down-regulating B cells from class switching to IgE production (Schaub et al 2006). Currently, the theory does not satisfy all conditions and circumstances and has met with mixed results in research (see Ring et al 2004). It is likely that the hypothesis will undergo further revision but still stands to synthesize the findings of many epidemiological studies (Linneberg 2008).

GLUTEN OVERVIEW

Proximal activation of CD, GA, and NCGS involve first-most the dietary intake of certain cereal grains- wheat, rye, or barley (and sometimes oats) (Freeman 2010). Each of these grains contains seed storage proteins that are responsible for adverse food reactions. There exist *four major classes of seed storage proteins: albumins, globulins, prolamins and glutelins* (Kagnoff et al 2007). Of the seed storage protein groups (Figure 5), prolamins contain the largest amount of known allergens to humans (Radauer et al 2007). Members of the wheat genus (Triticum) are known to contain prolamin- and glutelin- family proteins, termed gliadins and glutenins, respectively. Rye, barley and oats contain the prolamins secalin, hordein, and avenin respectively.

The list of known allergens occur almost entirely in a small subset of known protein families- of the 9318 currently recognized protein families, only 2% contain known allergens (Breiteneder 2008). Of those that do, most family members are non-allergenic- prolamins are an exception. All prolamins are characterized by a high proline and glutamine content (relative to other amino acids) and it is this high proline content that makes them difficult to digest (Shan et al 2005). Glutens are the only dietary proteins that seem to largely escape digestion by the gastrointestinal proteases (e.g. trypsin, chymotrypsin, and pepsin) and tend to gather in the small intestine (Hausch et al 2002).

Glutens (Latin for "glue") are peptide composites of prolamins and glutelins that reside naturally in the endosperm of grass related proteins and are also the components responsible for the rising and structuring of dough in bread-making. Wheat gluten (gliadin and glutenin combined) comprise 80% of the protein content of wheat seeds (Anjum et al 2007). In the three known gluten-averse conditions, persons can be hypersensitive to the prolamin or glutelin content of grains although glutenin (wheat glutelin) is the most common allergen. There are several subcategories of gliadins based on various biochemical properties (electrophoretic mobility and isoelectric focusing): a, b, γ, and ω-gliadins (Wieser et al 2008).

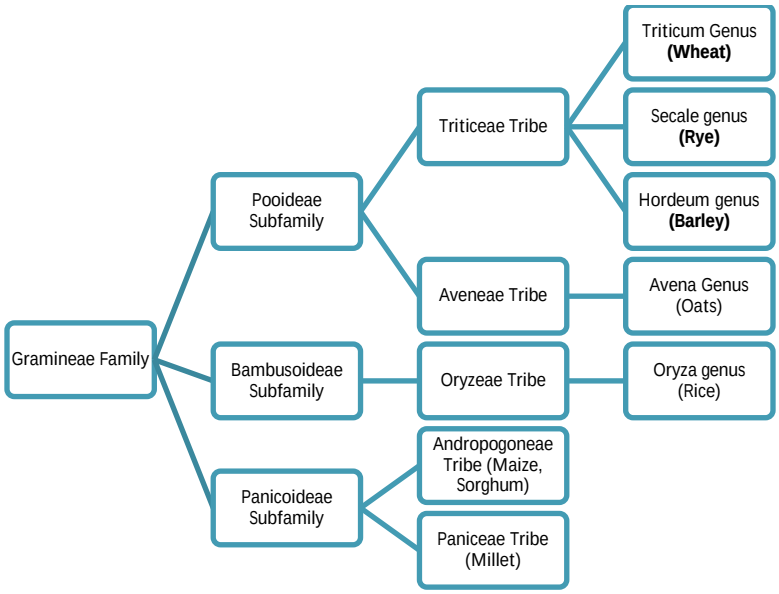

Figure 5. Taxonomy of Dietary Grains (adapted from Kagnoff et al 2007)

GLUTENS AS PATHOGENS

Glutens have been referred to as "non-replicative" pathogens (Bethune and Khosla 2008) because of their ability to induce disease. They are known to have adverse effects on the human digestive tract, regardless of genetic predisposition to CD (Drago et al 2006). A 2010 study by Biesiekierski et al utilized a double-blind randomized placebo-controlled setup to assess the effects of gluten on a group of healthy individuals prescreened for an absence of celiac disease (n=35). Both groups were assigned a gluten-free diet for 6 weeks and were also given 2 bread slices and one muffin per day. One group received their assigned baked goods with gluten and the other without (placebo). Those who received the gluten-containing food reported significantly different symptoms within a week of the trial: increased pain ($p=0.016$), bloating ($p=0.031$), and tiredness ($p=0.001$).

Zonulin-Signaling

The results of Bisiekierski et al (2010) might be explained by the fact that gliadin is largely recognized to increase permeability of the gut through the up-regulation of zonulin (Clemente et al 2003; Drago et al 2006; Lammers et al 2008). Zonulin is the only known reversible mediator of gut permeability- more specifically of the tight junctions that help form the barrier function of gut endothelial cells (Tripathi et al 2009).

Endothelial cells are key regulators of the body's internal environment; they make up the layers of the skin and line the GI and respiratory tracts, providing a barrier whose selective permeability dictates the transport/migration of various solutes and metabolites in to the body and also protects against invasion by various pathogens. Similar function is ascribed to the tight junctions (TJs) that bind these epithelial cells to one another (Koch and Nusrat 2009). Physically, these junctions (also known as zonula occludens) are anastomoses of scaffolding multi-protein complexes that anchor cytoskeletal elements (actin) between cells via cytoplasmic elements and the inter-cellular binding of many extracellular loops (Schulzke and Fromm 2009). TJs serve two larger goals: a 'barrier function' wherein they regulate paracellular flux of materials and also a 'fence function' that keeps various membrane proteins in the appropriate places (Sawada et al 2003). The nature of this system allows for regulation of tightness (permeability) and some channel formation through intra- and inter-cellular signaling. Proper function of this protein network is paramount to the avoidance of numerous disease states including inflammatory bowel disease, irritable bowel syndrome, food allergy, liver disease, type II diabetes and other auto-immune disorders, as well as many more (Groschwitz and Hogan 2009; Sawada et al 2003).

Zonula occludens toxin (Zot) was first isolated by Fasano et al (1991) from the bacterium Vibrio Cholerae. Its ability to reversibly disassemble tight junctions through the polymerization of actin immediately prompted the search for a eukaryotic analogue. In 2000, Wang et al reported the discovery

of human zonulin. The benefit of such a natural modulator is evident in the context of host-pathogen interaction: In the presence of bacterial invasion, it is advantageous to loosen tight junctions such that there is an efflux of water in to the intestinal lumen, and a flushing of pathogens from the system (Asmar et al 2002). However, this increased permeability is not without risk: the greater the increases in permeability, the larger the molecules are that can potentially pass through and interact the internal environment (including gut-associated lymphoid tissue) (Drago et al 2006; Fasano and Shea-Donohue 2005).

Immune System Effects

Not all glutens are created equal (Bateman et al 2004). Chains of varying lengths appear to interact differently with the immune system (Camarca et al 2009; Shan et al 2002). Several glutens (gliadins) are shown to be active contributors to CD (and potentially NCGS) (Hausch et al 2002). Gliadins type-α, ω and γ in particular seem to have the greatest stimulatory effect of T-cells (Camarca et al 2009). The α-gliadin 33-mer and p31-43 peptide fragments appear to be the most resistant to breakdown by tissue transglutaminase (mentioned in CD pathogenesis section) and thus the most pathogenic of the glutens (Jabri and Sollid 2006; Mazumdar et al 2010; Shan et al 2002; Thomas et al 2006).

There is some evidence that glutens may contribute to the development of autoimmune disease through a lowering of immune response thresholds (Nikulina et al 2004a). Dendritic cells of healthy mice exposed to chymotrypsin-treated gluten are stimulated to mature and upregulate production of MHC-II proteins and also to increases DC production of certain cytokines such as IL-1b, which is an important effector molecule of the inflammatory response. All observed effects were dose-dependent. Dendritic cells are especially concentrated in gut associated lymph tissue and are of primary importance in the induction of IgA responses (Rescigno and Di Sabatino 2009). Additionally, Horiguchi et al (2005)

reports a significant increase in NK activity in a small group of healthy persons when given wheat-containing foods (n=9, $p<0.05$), although no mechanism is suggested.

GLUTEN-AVERSE CONDITIONS

GLUTEN/WHEAT ALLERGY

Gluten Allergy (GA) is one of the most common allergies worldwide (Venter and Arshad 2011) (see Food Allergy Overview). It is an IgE-mediated (Type I) humoral immune response to ingested glutens (Boyce et al 2010). Symptoms are those of a 'classic' allergic reaction and may include gastrointestinal and/or cutaneous (skin) reactions. Gastrointestinal symptoms consist of vomiting, reflux, abdominal pain, and diarrhea. Cutaneous reactions may display as hives, angioedema, swelling of various tissues (e.g. hands, face, abdominal organs, and upper airway), and various forms of eczema. In severe cases, a person may go in to anaphylactic shock, which can be fatal if not treated quickly. Symptoms are similar to those listed but occur in extremes and are often accompanied by anxiety, difficulty breathing/swallowing/talking, and heart palpitations.

Gluten Allergy is more often diagnosed in childhood as are many IgE mediated hypersensitivities (Scibilia et al 2006). Additionally, timing of initial exposure appears to play a role in risk for gluten allergy development (Poole et al 2010). Children exposed to cereals after 6 months of age are more likely to develop GA than those exposed prior to 6 months of age (n=1612, $p=0.025$)

CELIAC DISEASE

Celiac Disease is an autoimmune disorder that likely falls under multiple categories of the Gell and Coombs (1963) Hypersensitivity Classification (Types 2 and 4). It has a strong genetic component, highly varied symptomology, and often occurs in conjunction with other medical conditions, autoimmune disorders in particular (Abadie et al 2011). Many researchers have attempted to characterize the non-

genetic factors that precipitate its development: breast-feeding (Guandalini et al 2007), infection (Fasano 2002; Mahon et al 1991, Plot and Amital 2008), intestinal dysbiosis (De Palma et al 2008; De Palma et al 2010; Di Cagno et al 2009), increased intestinal permeability (Abadie et al 2011; Bjarnason and Takeuchi 2009; Drago et al 2006) have all been considered. None of these suffices as a universal explanation and it is possible that development of CD should be considered on an individual level. Diagnosis of celiac disease is largely settled upon histological examination of the small intestine, antibody testing, and genetic screening (Kagnoff et al 2007). However, these methods are known to result in false negatives (histology) and false positives (antibodies and genetics) if not interpreted correctly, respectively (see Caja et al 2001; Tjon et al 2010). Below is a summary of CD epidemiology, current information on pathophysiology, hypersensitivity classification, genetic predisposition, a brief review of some potential non-genetic developmental factors, and a mockup of current known symptoms and comorbid conditions.

Epidemiology

Epidemiologic information of CD has traditionally focused on the marked prevalence in populations of European descent (Cataldo et al 2007; Tack et al 2010). As with allergy, estimates of epidemiology are plagued with varied testing criteria and comparison of results between studies is difficult.

Until the 1970s worldwide prevalence of CD was estimated at only 0.03% (Tack et al 2010). As of 2011, the consensus is that CD occurs in approximately 1% of adult populations worldwide (0.5-1.26% 95%CI) with the remaining exception of Japan (Dube et al 2005). Estimates vary somewhat by region (Table 8): Barada et al (2010) summarize epidemiological data from Middle East and Northern African populations (1950-2008). Data includes studies where diagnosis was confirmed using both serological tests and histological examination. Estimated prevalence for these regions falls between 0.14% in low risk populations to 44% in high risk populations. Here, low risk groups are healthy blood donors (mostly

men) and high risk populations are groups with type II diabetes, thyroid issues or chronic malabsorption issues. Shahbazkhani et al (2003) report a 1 in 166 prevalence of histologically confirmed CD in a group of health Iranian blood donors. Remes-Troche et al (2006) report 2.6% potential prevalence of CD in a group of healthy blood donors based on positive tTGA-IgA results (n=683, 68% men). Sood et al (2006) reports that 1 in 310 school age children (3-17 years) tested positive for CD based on serology and histology (n=4347) (see also Ramakrisha et al 2011). For yet unclear reasons, the Saharawi group of North Africa has the highest confirmed prevalence of CD by intestinal biopsy worldwide (Catassi et al 1999; Ratsch and Catassi 2001; Teresi et al 2010). Reported CD is still fairly absent from Japan, and other Far East countries (Freeman 2010) although there is some evidence that it may be present in China (Jiang et al 2009). Wu et al (2010) report a 2.6% prevalence in a high risk (type II diabetes or irritable bowel syndrome) prescreened group in China (n=78). Prevalence in the United States is estimated at 1:133 for low risk groups in the US (Fasano et al 2003).

Area	**Prevalence**	**Source**
North Africa (Egypt, Tunisia, Libya, Algeria)	0.14%-0.53% in "low risk" populations (mostly healthy men blood donors), 2.6-44% in high risk populations (persons with type II diabetes, thyroiditis or chronic malabsorption issues) 5.6% in the Saharawi	Barada et al 2010 Barada et al 2010 Catassi et al 1999; Teresi et al 2010
Europe (Czechoslovakia, Estonia, Finland, Hungary, Ireland, Italy, Norway, Portugal, Spain, Sweden, Switzerland, Netherlands, United Kingdom)	1 in 99 Finnish school children 2.4% Finland, 0.3% Germany, 0.7% Italy, adults ages 30-64 1:262 (Norway) – 1:85 (Hungary)	Maki et al 2003 Mustalahti et al 2010 Cataldo et al 2007
India	1 in 310 school children (~0.3%)	Sood et al 2006
Middle East (Turkey, Iran, Israel, Jordan,	0.33%-0.96% in "low risk" populations (mostly healthy men blood donors)	Barada et al 2010

Kuwait, Lebanon, Saudi Arabia, Iraq)	2.45-19% in high risk populations (persons with type II diabetes, thyroiditis or chronic malabsorption issues)	
Mexico	2.6% in a group of healthy blood donors	Remes-Troche et al 2006
United States	1:133 in healthy adults	Fasano et al 2003b

Table 8: CD prevalence estimates based on geographic region.

Diagnoses of CD are made at any age (Fernandez et al 2010). The bulk of cases are detected either in early childhood or ages 40-50 (Dube et al 2005). Adult diagnoses favor women at approximately age 40 and men at approximately age 50. Prevalence among children worldwide is 0.31%-0.9% (Hoffenberg et al 2003). Diagnosis in children more commonly isolates 'typical' CD presentation (Koluglu et al 2009). Up to 20% of patients are over age 60 at time of diagnosis (Saez 2006) and most of these people display the 'non-classic' phenotype with predominantly extra-intestinal manifestation or more subtle gastrointestinal symptoms (see Symptomology) (Fasano et al 2003; Nejad et al 2009; Vilppula et al 2008). Vilppula et al (2009) demonstrate an increasing prevalence of CD in the elderly in Finland from 2002-2005.

CD is more common in females (F:M= 1.8:1), a fact mostly attributed to HLA-DQ2 inheritance as a sex-linked trait from father to daughter (Khashan et al 2010; Megiorni et al 2008). Other non-HLA risk alleles may have a sex-linked bias and contribute to this difference but none has been described in the literature as of 2011. Tack et al (2010) has noted that immune-regulation also has a hormonal component that might play a role in differential distribution of CD between men and women. Interestingly, the CD sex ratio reverses for diagnoses in persons over the age of 60 (Green et al 2001).

Decker et al (2010) describe birth method as a potential risk factor for CD development. CD is more likely to develop in children delivered by Cesarean section than in healthy controls (p=0.014). Due to its genetic component, family members of CD-affected individuals are at higher risk for CD.

Concordance between monozygotic twins is 80%; Dizogotic twin concordance is only 11% (Nistico et al 2006). Risk for CD in first degree relatives of CD patients is 1:22 and 1:39 for second degree relatives (Fasano et al 2003). Hogberg et al (2003b) reports risk among 1st degree relative at 8.3%.

Celiac Disease is on the rise worldwide- a fact that is not entirely accounted for per improved diagnostics (Catassi et al 2010; Lohi et al 2007; Rubio-Tapia et al 2009; Tack et al 2010). Is likely that recent socio-cultural influences (since the 1970s) are key factors in this trend (see Non-Genetic Risk Factors and Hygiene Hypothesis).

Pathophysiology and Classification

Celiac Disease has been somewhat difficult to classify because it involves components of the innate immune system (the complement) as well as humoral and cell-mediated adaptive responses (Abadie et al 2011). Some have described Celiac Disease as an intestinal inflammatory disease (Atkins and Furuta 2010; Tjon et al 2010) but the most current consensus is that CD more closely resembles a T-cell mediated organ-specific autoimmune disorder (Abadie et al 2011). Ingested glutens that traverse the gut lining are met with a naturally occurring enzyme called **tissue transglutaminase (tTG)** (also known as TG2) (Figure 6) (Dieterich et al 1997; Elli et al 2009). Tissue transglutaminase exists largely in an inactive intracellular form that is released when its containing cell is damaged (Siegel et al 2008). It plays a role in breaking down glutens in a process called deamidation. Gorgun et al (2009) report that tTG is overexpressed in the mucosa of untreated CD. More specifically, the process changes non-charged glutamines into negatively charged glutamic acids (Dekking et al 2007). Per evolutionary chance this modification creates a molecule (a deamidated gluten or DAG) that is preferentially bound by APCs possessing a certain form of HLA-DQ2 or HLA-DQ8 molecule (Bethune and Khosla 2008). (The presence of HLA-DQ2 or –DQ8 molecules represents the largest genetic risk factor of CD development). The high affinity between deamidated gluten and tissue transglutaminase creates a situation where T-cells may

develop memory for either part of the DAG-tTG complex. T-cells can then alert B-cells to produce antibodies for the bound antigen. When the immune system begins developing antibodies for tTG (anti-tTG), an autoimmune disorder has developed and the individual can be classified as having Celiac Disease (Table 3). CD is the only autoimmune disorder for which the auto-antigen is known (Ferguson et al 2993). Antibodies produced against tTG and deamidated gluten (DAG) include IgA and IgG- classes (Caja et al 2011). This particular component of CD pathophysiology qualifies it for partial classification as type II hypersensitivity. In short, a self-perpetuating loop emerged: The presentation of glutens leads to the release of IFN-γ by T_H1, T_C and NK cells. IFN-γ upregulates expression of MHC-II molecules (HLA-DQ2 and –DQ8 included) which in turn increase presentation of gluten peptides to T_H1 populations. A secondary loop begins when tissue is damaged and tTG is further released. An increase in tTG increases the amount of deamidated glutens present which will in turn increase HLA-DQ presentation and thus the T-cell response.

Additionally, the involvement of CTLs and NK cells to attack 'infected' tissue qualify CD for type IV hypersensitivity classification (Anderson 1991). One hallmark of CD is the broad population expansion of **intraepithelial lymphocytes (IELs)** (Abadie et al 2011). Intraepithelial lymphocytes are the various lymphocytes positioned within the outer mucosal epithelial layers of the GI and reproductive tracts (Kindt et al 2008) that conduct immunosurveillance of the local epithelium. Most IELs are T-cells of various types. (Note: Below this mucosal layer is another layer called the lamina propria that contains mostly B cells, active helper-T cells, and macrophages.) IELs are different from other lymphocytes in that they release cytokines and attempt to destroy their targets immediately without need of priming. In other words, there is no difference between the naïve and activated forms. One of the most clinically dangerous aspects of Celiac Disease is the apparent destruction of absorptive surface of the small intestine resulting from this cell-mediated process. The small intestine is lined with villi (fingerlike projections) that increase the surface area dedicated to nutrient absorption. In most cases of untreated

CD, this lining is destroyed (villous atrophy and crypt hyperplasia). T_C IELs with a certain type of NK activating cell receptor (TCR-αβ+) have been linked to the destruction of the intestinal epithelial cells (Green et al 2003; Kutlu et al 1993). These particular receptors are thought to function in lowering activation thresholds for T-cells under various stresses (Bauer et al 1999). Another sub-type of T_C cell, TCR-γδ+ cells, is sometimes used as a marker for determining latent CD (Kaukinen et al 2007) (see Diagnosis). Epithelial cells that have come in to contact with the gluten or contain membrane bound tTG are marked for destruction because they are seen as infected. This destruction of the small intestine continues as long as glutens are ingested. Myrsky et al (2009) add that there is evidence of vascular remodeling in conjunction with proximal bowel damage.

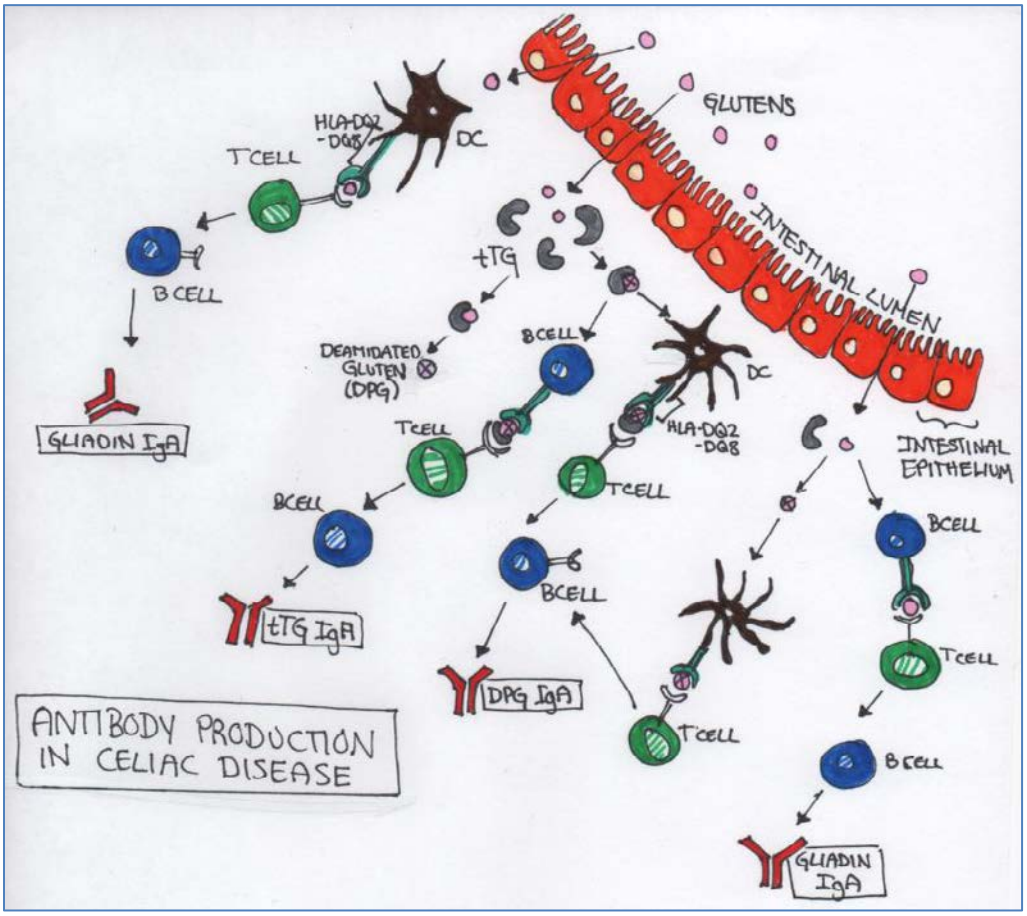

Figure 6. Antibody Production in Celiac Disease.

There is evidence that celiac patients also have autoantibodies for more than just tTG, although the symptomological implications of this have yet to be fully elaborated (Alaedini and Green 2008) (see Symptomology). These autoantibodies have received less attention in the literature because the presence of anti-tTG is the only known autoantigen to coincide with the acute onset of CD. Nonetheless researchers have somewhat variably reported autoantigens against a slew of other molecules: transglutaminase 3 (Lindfors et al 2011), cytoskeletal actin (Clemente et al 2000), ganglioside (Volta et al 2006), collagen, calreticulin, zonulin (Fasano 2000), factor XIII, synapsin I, cardiolipin, enolase α, and ATP synthase β chain (*see* Alaedini and Green 2008 for a review). It seems likely that further research will confirm that these additional autoantibodies to be associated with particular clinical manifestations of symptoms.

The pathogenesis of CD is far from fully elaborated. There is an apparent disconnect between outward gluten-averse symptoms and the presence of villous atrophy (Kaukinen et al 1998). Metabonomic analyses indicate that the physiological conditions of gluten-averse symptomology may precede observable destruction of the small intestine: Bertini et al (2009) characterized the metabolic signature of CD through proton mass spectroscopy. Serological analyses show that CD patients had lower levels of various amino acids, lipids, pyruvate and choline compared with healthy controls ($p<0.01$). Also, CD patients had elevated glucose (blood sugar) and 3-hydroxybutric acid, a protein created in the liver for energy use under conditions of low blood glucose ($p<0.01$). Urine analyses showed lower levels of mannitol, glutamate, glutamine and pyrimidines as well as higher levels of indoxyl sulfate (IS), choline, glycine, acetoacetate, uracil, mHPPA, and PAG ($p<0.05$). These serological results describe alterations of glucose metabolism, possibly through reduced glucose uptake by cells or errors in process of glycolysis. Urinalyses indicate differences in microbiota populations and an increase in gut permeability in CD relative to healthy controls (see Environmental Risk Factors). Bernini et al

(2010) has shown that this metabolic signature is similar between 'potential' and overt CD. Likely metabolic changes are in place before the onset of small intestinal destruction.

Interleukin-15 is also known to play a role in CD pathogenesis (Meresse et al 2004; Tang et al 2009): Harris et al (2010) report that monocytes from healthy individuals differentiated to macrophages through IL-15 exposure and subsequently produced IL-1b, IL-6, IL-15, IL-23, and TNF-a and also activated Th1 and Th17 cells. Monocytes from CD patients required a lessened exposure to IL-15 to produce the same effect in response to gliadin exposure, perhaps indicating lowered threshold activation. Manavalan et al (2010) confirms that persons with active CD and those being treated with a gluten free diet (with positive serology) both exhibit elevated levels of IL1b, IL-6, IL-8 and various Th2 cytokines. Meresse et al (2006) has shown that IL-15 may play a role in reprogramming CTLs into NK-like cells in CD and up-regulating the expression of MHC-I molecules. A comparison of IL-18 between active CD and healthy control showed mature IL-18 only to be found in CD patients (Salvati et al 2002), implying altered activity of T_H1 cells. The overexpression of tTG in CD may be due to an accumulation of particular gliadin fragments (p31-43 fragments) in the lysosomes of endothelial cells that trigger the epithelial cells to upregulate their tTG expression (Luciani et al 2010.

1. Destruction of lining of small intestine (crypt hyperplasia/villous atrophy).
2. Autoantibodies against tissue transglutaminase.
3. Population expansion of CTLs in mucosal lining (cytotoxic IELs) through lymphocytosis.
4. Overexpression of tTG in mucosal tissue.
5. Altered commensal bacteria in gut.
6. Altered IL-15 production alters T_H1 and T_H17 responses to gluten.

Table 9. Summary Physiological Features of Untreated Celiac Disease

The clinically significant findings of CD pathogenesis are summarized in Table 9. As of 2011, CD pathogenesis is best understood in a multiple hit model (Abadie et al 2011) wherein a secondary event is

needed to trigger development past predisposing genetics and physiology (Ferguson et al 1993). In addition to genetic preconditions, some form of intestinal stress must trigger IEL activation.

Genetic Components

CD has a strong genetic component. Numerous studies have attempted to characterize the genetic profile of CD, utilizing genome wide association studies, mapping and linkage analysis (Hunt and van Heel 2009). Approximately 90% of those diagnosed with CD carry the HLA-DQ2 haplotype, and the rest have the DQ8 haplotype (Dubois et al 2010; Sollid and Lie 2005) (6.23 odds ratio, Dubois et al 2010). Approximately 0.5% of persons affected by CD have neither haplotype (Cassinotti et al 2009). The presence of haplotypes also provides information on predisposition; CD associated HLA haplotypes appear to exhibit a dosage effect (Hernandez-Charro et al 2008). Persons who are homozygous for HLA-DQ2 are five times more likely to develop CD than those who are heterozygous. This homozygosity is also associated with the development of severe complications such as T-cell lymphoma (Cassinotti et al 2009).

Human leukocyte antigen- DQ are receptors found on antigen presenting cells (APCs) that are one of the many types of MHC class II molecules discussed earlier; they bind particular antigens and in turn expose them to helper T cells, which illicit the appropriate immune response (Jabri and Sollid 2006). DQs themselves are heterodimers (ab); they are comprised of two chains that are genetically coded by the HLA-DQA1 and –DQB1 alleles found on chromosome 6 (6p21.32). At present there are four known haplotypes associated with this heterodimer, two of which are considered to be high risk for CD (Cassinotti et al 2009). HLA-DQ2 (DQA*0501, DQB*0201, termed HLA-DQ2.5) and HLA-DQ* (DQA*03, DQB*0302)… are the more specific descriptions. Individuals homozygous for the HLA-DQ2.5 risk alleles are five times more likely to develop CD than those who are homozygous (Mearin et al 1983). The dose effect of the HLA-DQ2.5 risk alleles is directly related to the magnitude of T-cell response (Vader et al

2003). More recently Thomas et al (2009) failed to replicate the correlation of severity of CD symptoms with HLA-DQ2.5 based on endomysial antibody (EMA) testing (see Diagnosis).

The presence of HLA-DQ risk alleles appears to be required for CD development but is still not a good predictor - approximately 30% of the world population possesses these risk variants (Hunt et al 2008). The HLA-DQ2 risk allele is found more often in Western Europe, Northern and Western Africa, the Middle East and Central Asia, and the –DQ8 allele found slightly more often in Northern Europe and Central America (Cummins et al 2009).

Additionally, the genetic portrait of CD is not as simple as a bimolecular system. As of 2010, over 60 non-HLA risk minor loci have been identified in various genome-wide association studies (Amundsen et al 2010; Babron et al 2003; Forabosco et al 2009; Garner et al 2009; Greco et al 1998; Heap and van Heel 2009; Hunt et al 2008). Total risk explained by these non-HLA loci is estimated at 5% (Dubois et al 2010). Castellanos-Rubio et al (2010) have grouped many of these non-HLA loci according to physiological pathway and have found clusters pertaining to cell-cell communication, intracellular signaling, the ubiquitin-proteasome system, and regulation of the cell cycle, particularly apoptosis (cell death). One notable loci is CTLA4: there is some evidence to suggest that presence of two adenines at this locus is correlated with the presence of 'silent' CD (Gudjonsdottir et al 2009; Zhernakova et al 2005).

Environmental Factors

Environmental risk factors are of important consideration in pathogenesis given that genetics only account for approximately 30% of CD-risk (Freeman 2010; Greco et al 2002). Secondary precipitating factors may include: Intestinal dysbiosis (De Palma et al 2010), infection (Fasano 2002), and leaky gut (Groschwitz et al 2009). Ultimately, each of these risk factors involves a barrier defect of the intestinal epithelium, either temporary or long-term. This is because the first and foremost step in CD

pathogenesis is the inappropriate perambulation of the gut lining by glutens. The barrier function must be compromised to allow contact between IELs and glutens.

Immune Development and Intestinal Dysbiosis

De Palma et al (2010) confirm a shift in the population structure of gut commensal bacteria toward an increase in gram-negative relative to gram-positive bacteria in untreated children with CD compared with controls and CD-affected children on long-term gluten free diets (p=0.006 and p=0.045, respectively) (Table 10). Several of the bacterial populations measured were significantly decreased in children with untreated CD and one was increased. CD children treated with a gluten free diet showed values intermediate to those of the untreated CD and control groups in all cases of difference. Further analysis for presence of IgA showed that healthy controls had significantly higher IgA-coated Bacteroides-Prevotella compared with untreated CD (p=0.014). No difference in IgA coating of Bifidobacterium was observed between groups. Accordingly De Palma et al (2010) suggest a barrier defect present in CD. It is possible that reductions of favorable gram-positive populations in CD allow population expansion of gram-negative bacteria within the gut. On another note, children with allergies exhibit a delay in their development of Bifidobacterium and Lactobacillus (Kalliomaki and Isolauri 2003). Bifidobacteria are known to inhibit the inflammatory response that would otherwise be induced by gliadin exposure (Laparra and Sanz 2010).

Bacterium	**Untreated CD**	**Significance**
Bifidobacterium	Much lower	p=0.009
C. histolyticum	Lower	P=0.031
C. lituseburense	Lower	P=0.024
F. prausnitzii	Lower	P=0.045
Bacteroides-Prevotella	Greater	P=0.033
E.coli	Same	Not significant
Staphylococcus	Same	Not significant
Lactobaccillus-Enterococcus	Same	Not significant

Table 10. Intestinal Dysbiosis in children with Celiac Disease compared with healthy controls (from De Palma et al 2010).

Other research has supports that intestinal dysbiosis is present in CD although there are variations on the particular bacterium involved: Di Cagno et al (2009) report lactobacilli strains to be greatly reduced in CD. Ashorn et al (2009) highlight the presence of anti-Saccharomyces cerevisiae antibodies (ASCA) present in celiac patients that decrease with gluten free diet. Nadal et al (2007) describe a much reduced ratio of Lactobacillus-Bifidobacterium to Bacteroides-E.Coli in patients with CD (treated or untreated). They also found an overabundance of gram-negative bacteria relative to positive in CD patients. Sanz et al (2007) found that children with CD generally displayed a higher diversity of gut bacteria compared with healthy controls. However, Bifidobacteria species were more diverse in healthy controls. Similarly, Sanchez et al (2008) describe a reduced diversity of gut microbiota in CD children and also an increase in virulence factors for gut microbiota present. Papp et al (2009) found anti-microbial antibodies to be significantly higher in untreated CD than treated CD or healthy controls for antichitobioside (ACCA), antimannobioside (AMCA) and ASCA. Population structure of gut microbiota has been shown to influence intestinal permeability (Cinova et al 2011). Rubio-Tapia et al (2009b) report the presence of small intestine bacterial overgrowth (SIBO) in approximately 9.3% of CD patients, treated or untreated. Schippa et al (2010) detected a greater amount of Bacteroides vulgatus and E. coli in children with CD relative to controls and a generally higher biodiversity.

Breastfeeding appears to be protective against Celiac Disease (Guandalini et al 2007; Ivarsson et al 2002). Risk for celiac disease is greatly reduced for children for whom dietary gluten is introduced while still breastfeeding (Norris et al 2005). Also, gluten intermittently introduced with breastfeeding appears to correlate with the development of the non-classic CD phenotype (Guandalini 2007; Silano et al 2010). Breastfeeding affects the colonization of the gut by commensal bacteria- it is proposed that it reduces the risk of celiac disease through the encouragement of healthy immune development, which

depends on healthy colonization of the gut (Sanz et al 2008). Indeed, differences in the microflora of the newborn gut have been observed at one week of age between healthy and allergic infants, prior to any clinical manifestation of hypersensitivity (Bjorksten 2004). Rationale for the development of a disease state from intestinal dysbiosis ultimately derives from the hygiene hypothesis (Kalliomaki and Isolauri 2002).

Infection

Autoimmune diseases have been long observed to precipitate with infection (Plot and Amital 2008). The most commonly reported infectious associations of CD are Hepatitis C Virus (HCV) and Adenovirus 12 (Ad12). Published reports of CD association with Ad12 are limited (Kagnoff et al 1987; Mahon et al 1991). Mantzaris et al (1990) has suggested this to be a result of cross-reactivity between Ad12 and a-gliadin. More currently, HCV has been acknowledged for its CD association (Fine et al 2001; Ruggeri et al 2008). Hernandez et al (2007) did not find a reliable association between HCV infection and CD onset but notes that HCV may still be a precipitating factor in individual cases. Additionally, exposures to neonatal infections appear to be a reasonably good predictor of CD risk (OR 1.52) (Sandberg-Bennich et al 2007). Stene et al (2006) describe a possible increased risk for CD development following recurring rotavirus infection in early childhood. Notably, some researchers have suggested that certain infections may be protective against CD development (Plot et al 2009). Evidence of past infection (via antibody test) by rubella, cytomegalovirus (CMV) and Epstein-Barr Virus were all lower in Celiac patients as compared with healthy controls ($p<0.05$, $p<0.01$, $p<0.01$).

Leaky Gut

Increased intestinal permeability (IP), also known as 'Leaky Gut', is the most heavily investigated non-genetic factor in CD-risk to date (Abadie et al 2001; Bjarnason and Takeuchi 2009; Drago et al 2006;

Fasano 2011; Fasano and Shea-Donohue 2005; and many others). The reasons for this are two-fold: There is evidence that Celiac patients exhibit altered expression of epithelial junction proteins for tight junction formation in the small intestine (Ciccocioppo et al 2006) and the intestinal barrier function is known to be affected by a multitude of sources- exogenous factors, cytokines, and lymphocytes (Groschwitz 2009; Menard et al 2010). Currently increased intestinal permeability is largely acknowledged as a precondition for the development of CD (Visser et al 2009). Interleukins 4, 10, and 13 are known to alter gut permeability (Groschwitz 2009). T-cells modulate permeability through IFN-γ and TNF-a. Exogenous factors of IP include alcohol (Purohit et al 2008), heat stress (Yang et al 2006), non-steroidal anti-inflammatory drugs (NSAIDS) (Bjarnason and Takeuchi 2009; Kefalakes et al 2009; Kerckhoffs et al 2009), pathogens (Vibrio cholera, E. Coli, and Clostridium perfringens) (Fasano 1991, Muza-Moons et al 2004, and Fujita et al 2000, respectively), stress (Gareau 2008), anything that might cause low grade inflammation (Peuhkuri et al 2010), and others.

Altered zonulin signaling and increased permeability have been prime targets of CD genetic research (Monsuur et al 2005). So far, there are no genetic risk associations for up-regulated zonulin signaling in celiac disease, although the physiological relationship is observed (Wolters et al 2010): Drago et al (2006) exposed intestinal cell monolayers to gliadin and found that zonulin release was greater than in cells taken from CD than from a non-CD control group (0.67±.013 ng/mg and .02 ±.01, respectively, p=.01). CD patient cell groups showed elevated mucosal zonulin release at 5 minutes post-incubation that remained for one hour. The control group cells showed a slight increase in luminal secretion of zonulin that maxed at 15 minutes and subsided completely within half an hour. The same study found that chronic exposure of gliadin was able to decrease expression of key tight junction proteins (ZO-1 and occludin). Intestinal permeability issues in CD may be entirely environmentally mediated given that intestinal permeability appears to revert to normal parameters upon treatment with gluten-free diet (Duerkson et al 2005; Duerkson et al 2010).

A SPECTRUM OF SYMPTOMOLOGY

CD has a complex phenotype that is commonly split into 'classic' and 'non-classic/atypical', based on longstanding cultural construction by clinicians (Brown 1921; Nejad et al 2009; Torres et al 2007). Some groups have suggested that the 'atypical' form may actually dominate CD manifestation and consequently 'non-classic' as a division title has come into recent favor (Nejad et al 2009). Rampertab et al (2006) describe a shift toward diagnosis of non-classic phenotypes since the 1950s: prior to the 1980s the initial presenting symptom was diarrhea in over 90% of CD patients. After 2000, less than 40% of persons diagnosed with CD initially present with diarrhea (Lo et al 2003). Rawal et al (2010) report similar findings. It stands to be seen whether this change is attributable to changes in diagnostic processes but Rampertab et al (2006) suggest that sociocultural trends such as breastfeeding practices may be a factor. Lastly, there is evidence to suggest that periods of gluten intake and withdrawal over time may change the phenotype of CD within the same individual (Kurppa et al 2008).

Below is a basic outline of known CD symptoms (Table 11):

Classic Symptoms	**Non-Classic Symptoms**
Abdominal pain	Alopecia
Anemia*	Arthritis
Anorexia	Dermatitis herpetiformis
Constipation*	Delayed Puberty*
Diarrhea	Depression
Foul-smelling stool	Dyspepsia
Fatigue	Hepatitis
Vomiting*	Infertility
Weight loss	Multiple miscarriages
Short stature	(women)
Irritability	Migraines
	Obesity
	Iron deficiency

Table 11. Division of some CD symptomology in to classic and non-classic categories (as listed by Nejad et al 2009 and Torres et al 2007). *Classification of symptom differs between sources.

The difference between classic and non-classic CD (as listed here) is the presence/absence of gastrointestinal symptoms (Nejad et al 2008; Torres et al 2007) (Table 11). Sources disagree on the exact symptom division of these two groups. Furthermore, patients with classic CD commonly exhibit non-classic symptoms (Torres et al 2007) and many have only these non-classic symptoms. Confusingly, persons without classic symptoms have been referred to as 'asymptomatic' or 'silent' (Ferguson 1993; Tack 2010) despite the clear presence of clinical manifestation. Instead, it appears that a spectrum of phenotypes exist (Tack et al 2010) and CD may be better illustrated by symptom grouping based on affected organ/tissue (as in Dickey 2009), with recognition that individual manifestations are diverse, likely from variability in pathogenesis (Table 12). Gastrointestinal issues can be sub-grouped into those pertaining to malabsorption and dysmotility (Tursi 2004).

GASTROINTESTINAL SYMPTOMS	
Malabsorption Issues	
Diarrhea, steatorrhea	
abdominal cramps, bloating	
Weight loss	
Dysmotility Issues	
Vomiting	
Epigastric pain	
Constipation	
Heartburn	
NON-GASTROINTESTINAL SYMPTOMS	
Hematological Symptoms	
Iron, B12 and/or folic acid deficiency	Paul et al 2010
Anemia	Harper et al 2007
Hyposplenism	Bullen et al 1980
Liver-related Symptoms	
Abnormal liver chemistry	Rubio-Tapia and Murray 2007
Skin/Mucous Membrane Symptoms	
Dermatitis herpetiformis	Marietta et al 2007
Alopecia areata	Neuhausen et al 2008
Aphthous mouth ulcers	Malahias et al 2009
Rheumatological Symptoms	
Arthritis	Zhernakova et al 2011
Bone-related Symptoms	
Osteoporosis	Bianchi et al 2008
Osteomalacia	Jafri et al 2008

Tooth enamel defects	Malahias et al 2009
Hypocalcaemia	McNicholas and Bell 2009
Increased fracture risk	Bianchi et al 2008
Gynecological Symptoms	
Late puberty/late menarche	Bona et al 2002
Infertility	Freeman 2010b
Recurrent miscarriages	Anjum et al 2009
Neurological Symptoms	
Cerebellar ataxia	Burk et al 2001; Hadjivassiliou et al 2003, 2006
Partial seizures/epilepsy	Freeman 2008; Hadjivassiliou et al 2010
Migraines	Zelnik et al 2004
Peripheral neuropathy	Alaedini et al 2002
Other/Mixed	
Chronic Fatigue	Jorda and Vivancos 2010
Anxiety and/or depression	Collin et al 2008; Hauser et al 2010
Short Stature	Troncone and Kosova 2010
Colon polyps	Casella et al 2010
Selective IgA deficiency	Collin et al 1994

Table 12: Known CD symptomology sorted by affected tissue/organ system and relevant citations. Suggested readings are provided for 'non-classic' symptoms.

Hematological Symptoms

Iron, B12 and folic acid are common deficiencies in CD and many persons are anemic at time of diagnosis (see Halfdanarson et al 2007). Other hematological associations have been noted: thrombocytosis, thrombocytopenia, leukopenia, venous thromboembolism, and hyposplenism (Bullen et al 1980). Anemia has generally been considered an effect of malabsorption in CD but recent work by Harper et al (2007) suggests that this is not the case and that inflammation must also be involved. Current hypotheses include absorptive issues of iron, increased blood loss in feces, and anemia of chronic disease (mediated by pro-inflammatory cytokines) (Hersko and Patz 2008). Hershko and Patz (2008) summarize the current findings of CD and anemia: As many as 50% of persons detected by various population screenings have been found to be anemic. Approximately 5% of persons who exhibit iron deficiency anemia have been found to have CD.

Liver-related Symptoms

Rubio-Tapia and Murray (2007) conducted a review of current research discussing liver-related symptomology of CD. Hypertransaminasemia is present in approximately 40% of adults and 54% of children with CD upon diagnosis. CD associated with a 2-6x risk for liver disease. The underlying physiology of this connection is unknown. Autoantigens for tTG have been reported in the liver and it is proposed that there is a proximal activation of the inflammatory response that results in damage to the liver.

Skin-related Symptoms

Dermatitis herpetiformis (DH) is considered the cutaneous manifestation of CD that occurs in 4.5-33% of CD (Collin and Reunala 2003; Collin et al 1994; Marietta et al 2008). Epidermal transglutaminase (TGe) is closely related to tTG, both of which are considered autoantigens in DH (Marietta et al 2008). The reasons for this connection remain unclear (Marietta et al 2008). Several other skin conditions associated with CD appear more commonly in those who also display DH: oral mucosal lesions, alopecia areata (Neuhausen et al 2008) and vitiligo. Aphthous mouth ulcers are significantly associated with CD and Malahias et al (2010) have suggested clinicians to examine the mouth when diagnosing CD.

Bone-related Symptoms

Several bone-related conditions are associated with celiac disease: low bone mineral density (BMD), hypocalcaemia, osteopenia, osteoporosis, and osteomalacia (see Bianchi et al 2008 for review). The connection likely lies in the metabolic alterations present in CD that in turn affect bone and mineral metabolism. The two most tenable hypotheses involve the increased release of pro-inflammatory cytokines in CD as a result of chronic inflammation and nutrient malabsorption (such as calcium). Low BMD can be directly linked to malabsorption issues and osteoporosis (and osteomalacia) is purported to

come from a decrease in calcium uptake (or possibly vitamin D deficiency) that leads to the development of an overactive parathyroid (hyperparathyroidism). However, hyperparathyroidism appears to be rare in CD and limited to case reports (Collin et al 2002). Additionally, leptin plays a role in bone remodeling and correlates with BMD (Hamrick and Ferrari 2008). Leptin levels are reduced in children with CD (Ertikin et al 2006). Jafri et al (2008) have suggested that earlier detection of CD will help reduce the risk of fractures form bone related changes. Dental enamel defects (linear enamel hypoplasia) have been significantly correlated with CD and can be physiologically attributed to early onset CD (prior to or during enamel formation) (Malahias et al 2010). Rashid et al (2011) reviews the oral manifestations of CD and encourages dentists to help play a role in CD diagnosis, as enamel defects and mouth ulcers may be the only outward manifestations of CD in some individuals.

Gynecological Symptoms

Some women with undiagnosed CD experience frequent miscarriages (Sheiner et al 2006). Tissue transglutaminase is widely expressed in placental tissue (Robinson et al 2007), the function of is not fully elucidated. Anjum et al (2009) report the appearance of IgA-tTG in tissue staining along the outermost maternal-facing layer of the placenta (syncytiotrophoblast plasma membrane) that may reduce placental function. Approximately 4-8% of women with unexplained infertility have been subsequently diagnosed with CD (Meloni et al 1999). Freeman (2010b) has suggested that CD might be provoked by exposure to fetal antigens during pregnancy.

Neurological Symptoms

Approximately 10% of persons with CD also suffer from a neurological disease- especially peripheral neuropathy and cerebellar ataxia (Burk et al 2001; Willis and Hovell 1996). Opthalmoplegia, epilepsy, encephalopathy, myopathy, Stiff-man syndrome, neuromyotonia, myelopathy, and dementia have also

been observed (Hadjivassiliou et al 2010). Anti-ganglioside antibodies present in patients with celiac disease correlated with the presence of peripheral neuropathy (Alaedini et al 2002). CD patients screened for serum anti-ganglioside displayed distal sensory loss. Alaedini et al (2007) suggest that some neurological disorders associated with CD may be due a cross-reactivity of anti-gliadin antibodies to neuronal synapsin I. However, anti-synapsin antibodies are observed in CD cases with and without neurological symptoms.

Gluten-related ataxia is the most common cause for sporadic idiopathic ataxia, based on anti-gliadin antibody testing (20% of all ataxia) (Pellecchia et al 1999; Hadjivassiliou et al 2003). A sample of 224 ataxia patients in the UK have shown that only 13% presented with gastrointestinal symptoms but over 70% were positive for the HLA-DQ2 risk allele. A more recent study by Hadjivassiliou et al (2006) observed widespread deposition of IgA-tTG in the cerebellum, pons and medulla of patients with gluten ataxia that was absent in non-gluten ataxia. Freeman (2008) reviews the possibility that some neurological symptoms may be the result of vitamin deficiencies: Vitamins B1, B6, B12, E, and niacin deficiencies are all known to be associated with neuropathy. B1, B12 and niacin are known to be associated with cerebellar ataxia.

Depression and anxiety are common in CD (Collin et al 2008). There is evidence that anxiety does not completely resolve with gluten free diet (Hauser et al 2010).

Other Symptoms

Casella et al (2010) analyzed a group of adults with CD over the age of 40 on gluten free diets (n=42) for abnormal colonoscopic findings. Polyps, diverticula, and inflammatory changes presented in 26% of patients. Lymphocytic colitis, malanosis coli, rectal histiocytosis were found in 36% of participants. The control group exhibited none of these symptoms. Additionally, persons with CD showed an elevated

presence of eosinophils compared with controls. It is suggested that colonoscopies be performed in subjects suspected of CD.

Comorbid Conditions

A host of additional conditions have appeared in conjunction with CD (Table 14). Note that the distinction between comorbidity and clinical symptomology of CD is difficult because there is still insufficient research to explain extra-intestinal manifestations, as demonstrated by the continued disagreement between classic and non-classic symptoms (Alaedini and Green 2005). Given the multi-systemic nature of CD, it is possible that some of these 'comorbid' conditions may be instead etiologically related. The following conditions have been described as comorbid with CD:

CONDITION	Suggested Reading
Addison's Disease*	O'Leary et al 2002
Autoimmune Gastritis*	Stancu et al 2001
Autoimmune Hepatitis*	Tovoli et al 2010
Autoimmune Thyroid Disease*	Elfstrom et al 2008
Autism	Vojdani et al 2004
Behçet Disease*	Zamani et al 2009
Dermatomyositis*	Song et al 2006
Eczema*	Collin and Reunala 2003
Fibromyalgia	Zipser et al 2003
Irritable Bowel Syndrome* (Crohns Disease)	Casella et al 2010
Lactose Intolerance	Ojetti et al 2005
Lupus Erythematosus*	Mirza et al 2007
Multiple Sclerosis*	Rodrigo et al 2011
Myasthenia Gravis*	Tack et al 2010
Non Hodgkins Lymphoma	Goldacre et al 2008
Peptic Disease	Levine et al 2009
Primary Biliary Cirrhosis*	Rubio-Tapia et al 2007
Primary Sclerosing Cholangitis*	Hay et al 1988
Psoriasis*	Collin and Reunala 2003
Schizophrenia	Cascella et al 2009
Sjögren Disease*	Collin et al 1994
Small Intestinal Cancer	Elfstrom et al 2011

Type 1 Diabetes Mellitus*	Frisk et al 2008
Vitiligo*	Tack et al 2010

Table 13. Conditions that have been described as comorbid with celiac disease. Asterisks (*) are placed next to autoimmune conditions. Note: This list is not exhaustive, but represents some of the commonly cited findings for comorbidity.

Autoimmune Conditions

Many conditions comorbid with CD are autoimmune disorders (Somers et al 2006; Somers et al 2009). Many autoimmune conditions not listed here have been reported in conjunction with CD. This is entirely consistent with the observation that multiple autoimmune conditions tend to appear both within individuals and families (Cooper et al 2009). As much as 25.6% of participants with CD display one or more additional autoimmune disorders (Bardella et al 2009, n=297). Part of the explanation may be that HLA-DQ2 and -DQ8 risk alleles are associated with numerous autoimmune conditions (Kumar et al 2001).

Autoimmune thyroid conditions and type I diabetes are some of the most commonly comorbid conditions for CD. CD, autoimmune thyroid disorders, and AIDDM all share the HLA-DQ2 risk allele (Kumar et al 2001). CD occurs in approximately 4.1% of diagnosed autoimmune insulin-dependent diabetes mellitus (AIDDM) (Collin et al 2007). This number is based on a prevalence synthesis of 40 screening surveys conducted between 1987 and 2001 (n=16194 adults and children). Persons with both CD and AIDDM tend to mild to no gastrointestinal symptoms. Some have suggested the two conditions to be resultant of each other, a claim that is supported by mutual increased risk of subsequent development for the second condition (Kumar et al 20010. CD and AIDDM have other shared risk alleles apart from HLA-DQ: RGS1, IL18RAP, and TAGAP (Smyth et al 2008). Thyroid disorders are found in approximately 5% of person with CD (Collin et al 2007). Elfstrom et al (2008) found significant association between CD and subsequent hypothyroidism (Hashimoto's Thyroiditis), thyroiditis and hyperthyroidism (Grave's Disease) (hazard ratio= 4.4, 3.6, 2.9, respectively) and that these risk values

were increased slightly in children ($p<0.001$). Thyroid disease and subsequent CD is also observed for all three of these thyroid conditions ($p<0.001$).

Other autoimmune disorders have similar comorbidity rates: Sjogren's Syndrome is found in 3.3% of CD compared with 0.3% in health controls (Collin et al 1994) ($p=0.0059$). A screen for CD within a group of patients with Addison's disease (AD) found a prevalence of 12.2% (n=46) (O'Leary et al 2002). Dermatomyositis has been reported in co-occurrence with CD only three times in the literature up through 2006 (Song et al 2006). It is reported to respond positively to a gluten free diet. Similarly, autoimmune gastritis is very rare but has been reported in conjunction with CD and noted to improve with gluten-free diet (Stancu et al 2001).

Diagnosis

Diagnostic procedures for CD may include histological examination, serological testing, genetic testing, treatment with gluten free diet, as well as basic symptom observation (Dickey 2009). Typically diagnosis is based off positive serology and histology. Clinicians commonly choose to conduct serological tests prior to scheduling a duodenal biopsy (Kelly 2004) unless there is a high clinical suspicion of CD (Green 2009).

Serological (blood) testing is one of the least invasive and affordable procedures used in CD diagnosis. For adults, the best serological tests look for antibodies to tTG and deamidated gluten peptides (DGP) (Hill 2004). Anti-gliadin (AGA) and anti-endomysium (EMA) appear to be better for children under the age of 2. Tests for IgG antibodies are commonly used in situations where a patient has selective IgA deficiency (Prince et al 2000; Vermeersh et al 2010). Note: Persons with selective IgA deficiency are 10x more likely to develop CD (Collin et al 1994). Two of the important factors of testing are the **sensitivity** and **specificity** values for the test, which are the percentages of true positives and true negatives, respectively (Table 14). Sensitivity and specificity values are also known to fluctuate

based on age, CD phenotype and degree of histological damage: Abrams et al (2006) analyzed the sensitivity and specificity for various anti-tTG antibody tests used by two popular commercial labs and found significant variability in these values. Sensitivity in particular is lower for patients with only partial villous atrophy compared with those demonstrating total villous atrophy (42.3% versus 90.0%, $p<0.0001$). (Freeman 2004; Hopper et al 2007). Anti-gliadin testing has been largely abandoned due to low sensitivity (Armstrong et al 2011). No serological test for CD is perfect (100%) for either of these parameters and they are generally used in combination with other diagnostic methods.

Test	Sensitivity	Specificity	Accuracy
IgA-tTG	78%	98%	90%
IgA DGP	74%	95%	86%
IgG DGP	65%	98%	84%
IgG + IgA DGP	75%	94%	86%
IgA AGA	63%	90%	79%
IgG AGA	78%	98%	90%

Table 14. Sensitivity and specificity values of various serological tests for CD in adults (values from Rashtak et al 2008).

Histological exam has long been considered a "gold standard" in CD diagnosis (Dickey 2009; Freeman et al 2011). In this procedure, a small biopsy is taken from the duodenum and examined for evidence of villous atrophy and crypt hyperplasia as well as IEL lymphocytosis (an influx of IELs to the region). Results are most often scored based on the Marsh-Oberhuber Classification (Marsh 1992; Oberhuber et al 1999): (0) "Latent CD"- Abnormal serology tests but no IEL lymphocytosis or villous atrophy present; (1) "Lymphocytic enteritis"- Normal villi with some IEL lymphocytosis; (2) IEL lymphocytosis plus some villus atrophy; (3) Partial, near total or complete villous atrophy. Histological exams must be carefully interpreted: Biopsy analysis is somewhat subjective and is known to vary between pathologists (Corazza et al 2007). Gastric lymphocytosis criterion for CD is approximately 25 IELS/ 100 epithelial cells (Carmack et al 2009). Villous atrophy and crypt hyperplasia are known to occur in other disease states

including tropical sprue, giardiasis, autoimmune enteropathy as well as HIV enteropathy (Green and Jabri 2006). Lastly, degree of intestinal damage does not correlate well with severity of symptoms (Brar et al 2007; Murray et al 2008). Some researchers have described an over-reliance on histological testing in clinical settings: Mohamed et al (2008) re-analyzed 14 patients who had failed diagnosis for CD, based on the Marsh-Oberhuber classification, by using a test for antibodies (CD2, CD3, CD 7, CD8, CD69, and Ki67) indicative of mucosal damage. Nine of these patients qualified for CD diagnosis. Measurement of TCR-γδ+ cell levels has been recently shown to be a better detector of latent CD than the Marsh I classification (Kaukinen et al 2007).

Genetic testing for the HLA-DQ2 and –DQ8 risk alleles are becoming more common use, as the cost of genetic testing decreases (Green and Jones 2010). Note that these tests can only report on a genetic predisposition that is required for CD development- not if the person has manifested CD.

Diagnostic Models

Phenotype labels for CD have been generated to fit current diagnostic procedures and the expanding spectrum of diagnosed CD (Troncone et al 2004): "Latent/potential" CD indicates positive serology only. "Asymptomatic/silent" persons have a positive serology and histology for CD but lack classic symptomology. "Symptomatic" express all three conditions. Despite these labels, researchers and clinicians have had some difficulty in generating a cohesive model for diagnosis; many diagnostic procedures, even if used in conjunction, fail to catch the full clinical spectrum of CD. A 2009 study by Tursi et al recruited 549 patients with CD for retrospective assessment. Less than half (47.7%) qualified for classic/symptomatic description. Subclinical CD was in the slight majority with 47.7% and 6.6% fell under the silent classification.

Ferguson et al (1993) was the potentially the first to suggest that a secondary factor is required to exacerbate underlying genetic predisposition into disease development; Weinstein et al 1974 was the

first to suggest the existence of a 'proceliac' state. Latent celiac disease has also been referred to as subclinical, silent, occult celiac disease, low-grade enteropathy, potential celiac disease, gluten sensitivity with mild enteropathy (summarized in Ensari 2010).

One of the largest pitfalls of current diagnostic procedure is the observation that the causes of small intestinal destruction appear to be independent of the development of autoantibodies (Abadie et al 2011) (see Pathogenesis). In attempt to ameliorate continued missed diagnoses, Catassi and Fasano (2010) have supplied a simple "4 out of 5" model to be used in CD diagnosis (Table 15). As long as four of the five criteria is met, the patient should qualify for CD diagnosis. If genetic testing is not performed, the criteria should be followed as 3 out of 4.

1. Presence of "Classic" Symptoms
2. Positive autoantibody IgA test.
3. HLA-DQ2 or –DQ8 genotype
4. Presence of enteropathy upon histological examination
5. Positive response to gluten free diet

Table 15. "Four out of Five CD Diagnosis Model"

Positive response to a gluten free diet cannot be taken as a proof-positive for CD: Campanella et al (2008) believe that positive response to a gluten free diet should not be primarily used in CD diagnosis because abdominal symptoms post-cereal ingestion are not entirely limited to CD (Kaukinen et al 2000). They re-analyzed approximately 180 patients put on gluten free diets due to CD diagnosis and found that only 51 qualified for diagnosis on the basis of histology and serological markers.

Treatment of CD is still limited to a strict gluten-free diet (Schuppan et al 2009), although many researchers have attempted various therapeutic strategies (*see* Lerner 2009 and Sollid and Lundin 2009 for reviews).

Complications of Celiac Disease

Approximately 2-5% of CD patients are considered refractory and will not recover when put on a gluten free diet (Tack et al 2010). **Refractory CD** can be subdivided in to Types I and II based on cytometric analysis of IELs (Daum et al 2005). Type I is characterized by a normal T-cell population and responds well to steroid treatment. Type II refractory CD T-cells are abnormal, changes of which appear to be associated with a TCR gene rearrangement and recurrent trisomy for chromosome 1q (a section of the first chromosome appears in triplicate instead of duplicate). Prognosis of type II RCD condition is poor because there is a greatly increased risk for development of malignancies. The 5 year survival rate for type I is 80-96%, but only 44-58% for type II (Biagi et al 2010). Type II refractory CD with enteropathy-associated T-cell lymphoma has a survival rate of only 8%.

Many studies have described an increased risk for non-Hodgkin lymphoma in untreated CD (3-6 fold risk) (see Elfstrom et al 2011). Two types of lymphoma are notably observed in refractory CD patients: enteropathy-associated *T-cell* lymphoma (EATL) and enteropathy-type *intestinal* T-cell lymphoma (EITCL) (Farstad and Lundin 2003). As many as half of all Type II refractory CD patients will develop one of these lymphomas (Al-toma et al 2007). Factors that contribute to the development of refractory sprue include *poor gluten-free diet compliance and delayed diagnosis* (Freeman 2009). Silano et al (2007) found that patients diagnosed with CD at a later age were at higher risk for developing neoplasms compared with those diagnosed at earlier ages (n=1968). They utilized the Italian Registry of Complications of Coeliac Disease (est. 1982) to look for subsequent tumor development in persons diagnosed with CD at various collaborating centers in Italy from 1982 to 2005. Standardized incidence ratio for all cancer was 1.3 (95%CI: 1.0-1.7, P<0.001). SIRs for specific cancer forms were: non-Hodgkin lymphoma 4.7 (95% CI: 2.9-7.3, p<0.01), small bowel adenocarcinoma 25 (95% CI: 8.5-51.4, p<0.001), Hodgkin lymphoma 10 (95% CI: 2.7-25, p=0.01), and stomach carcinoma 3 (95% CI:1.3-4.9, p<0.08).

Mean age for persons with CD who were diagnosed with cancer was higher than those who did not develop cancer (47.6±10.2 years versus 28.3±18.2 years) ($p<0.000$).

Malignancies are not the only complications of CD: Tursi et al found 3.3% of CD patients on gluten-free diets developed complications that also included secondary autoimmune disease, myocardial infarction, and recurrent miscarriages.

Vitamin B deficiencies are of notable concern for those on a gluten-free diet (Thompson et al 2005). Hallert et al (2009) conducted a double-blind placebo-controlled study of B-vitamin supplementation in CD patients for 6 months (n=65). Participants were assigned to 2 groups: one receiving daily doses of folic acid (B_9) (0.8 mg), cyanocobalamin (B_{12}) (0.5 mg), pyridoxine (B_6) (3mg) and the other a placebo. Vitamin-B and homocysteine levels as well as psychological well-being were measured post-experiment and it was found that persons receiving vitamin supplementation had significantly improved well-being ($p<0.01$) with reduced anxiety and depression ($p<0.05$ each).

NON-CELIAC GLUTEN SENSITIVITY

In March 2011, Sapone and colleagues published the *first* article to confirm "Gluten Sensitivity" (NCGS) as a condition separate from CD: Gluten-sensitive, celiac, and control groups were enrolled in a gluten challenge under clinical supervision for four months (n=26, 42, and 39 respectively). Post challenge they underwent serological, genetic and histological analyses for markers of CD. Post analysis they were put on a gluten-free diet and monitored for improvements. Here, gluten sensitive individuals are those who fail to display positive serology for autoantigens and do not have histological damage yet still do poorly on gluten-containing diet. Many symptoms overlap CD and GS, although GS symptomology appears to be less severe.

Sapone et al (2011) found that GS symptoms improved more quickly on GFD (days). Half of NCGS patients (48% were positive for anti-gliadin antibodies (AGA) and over half displayed the HLA-DQ2

or –DQ8 markers. AGA presence and HLA markers did not correlate. Persons with CD had significantly increased intestinal permeability relative to NCGS patients (p=0.0138). NCGS patients actually had better barrier function (per lactulose/mannitol urinary ratio) than healthy controls (p=0.0308). Expression of tight junction protein claudin 4 (CLDN4) was found to be higher in NCGS relative to either CD or healthy controls (p=0.0286). (CLDN4 is thought to decrease tight junction permeability). Autoimmune antibodies were not found in the NCGS patients or controls. Bowel biopsy for NCGS showed at most minor affect. NCGS patients displayed higher levels of IELS relative to normal ranges (still lower than those in CD). Cytokine IL-6 levels were significantly elevated in CD but not in NCGS. NCGS patients also showed a reduced expression of IFN-γ.

Changes in IELs and the presence of AGA suggest the involvement of the adaptive immune system, although to a lesser degree as compared with CD. Given the increase in IL-6, NCGS may be an inflammatory response generated primarily through innate immune system pathways. The results of this study largely confirm the hypotheses from prior work from the same group (Sapone et al 2009). However, further research is needed to better characterize the etiology of this condition.

METHODS

PROJECT GOALS:

Previous research indicates that persons affected by CD in US often feel that diagnosis was too delayed (Green et al 2001) (n=1612, most diagnosed at ages 40-50). Current data shows that average delay for CD diagnosis in the United States is approximately 11 years (Fasano et al 2003). Such delays may reflect the lack of information and relatively low importance of food allergies from general practitioners and some internists (Gupta et al. 2008, 2009, 2010).

The goal of this research is to characterize the diagnostic trajectories for gluten-averse conditions and make suggestions for decreasing time to diagnosis.

SURVEY DESIGN

A questionnaire was generated, modeled partly after Cranney et al (2003) and Green et al (2001) (Appendix I: Gluten-Averse Condition Survey). The local Bellingham Gluten Intolerance Group (BGIG) assisted in the construction. A subset of the questionnaire was used for this project:

- **Background Information**: age, sex, height, weight, education, marital status, occupation, and income.
- **Personal Path to Diagnosis**: breastfeeding, travel outside the US and Canada, frequency of food related illness in the US, initiation of diagnostic process, medical professionals consulted, timeline of diagnosis, testing performed, final diagnosis, and if first in family to be diagnosed.
- **Symptom Inventory:** on Lickert scale for before diagnosis and after diet change.
- **Additional Presenting Diseases/Conditions:** for participant and family (mother, father, brother, sister, grandparents, children and grandchildren.

PARTICIPANT SAMPLING

Participants recruited through BGIG and also using an on-line solicitation (Survey Monkey) and snowball sampling. Both affected individuals and "friends" of affected individuals that are the same sex and close to the same age (controls) filled out the same questionnaire (copies attached). Here, only the results of the affected individuals were used.

STATISTICAL ANALYSES

All statistical analyses were performed using SPSS 19.

ETHICS

The questionnaire and sample design were approved for an exemption by the Human Subjects Review Committee at Western Washington University.

RESULTS

CHARACTERIZATION OF SAMPLE

A total of 35 individuals responded to the survey (n=35).

Age

Range of 21-89 years, mean = 57.02 +/- 14.42 years, median = 59 years.

Sex

Females made up the majority of the sample (n=32); only 3 males in the sample.

Menopause Status

Of the women in the study, 21.9% were premenopausal (n=7), 15.6% were peri-menopausal (n=5), and 62.5% were post-menopausal (n=20).

Ancestral origins

All participants who responded to this section were of mixed European ancestry (n=33). Two individuals did not respond on this question.

Family income

Family income sections were answered by 31 participants. Twenty reported incomes over 50K (57.1%), 8 reported incomes over 100k (25.8%).

Marital Status

All responded to marital status: 74.3% were married (n=26), 5.8% were widowed/widowers (n=2), 14.3% were single (n=5), and 5.8% were cohabiting with mate (n=2).

Years of Education

All participants responded to the education questions: All but 1 indicated that they had attended at least some college classes. Approximately 28.6% indicated education through graduate school (n=10). Twenty-five participants reported on their particular training: 28% of those had bachelor's degrees in the hard sciences (biology or math/computers) (n=7), 33.3% had bachelor's degrees in the social sciences (n=12), and 16.7% had humanities degrees (n=6).

PATH TO DIAGNOSIS

All quotes are provided with anonymous participant number (e.g. P29).

Age at Diagnosis

All participants provided approximate age at diagnosis. Average age at diagnosis was 49.7+/-13.2 years (median=53), range of 19-77 years. Age at diagnosis for persons who identified as 'gluten intolerant' was 50±13.8 years (median = 48). For persons with CD, average age at diagnosis was 51.8 ±12.2 years (median = 55).

Initiation of diagnosis (Who)

Open ended question: "Can you supply any other details about the context that started the diagnostic process?"

Over half of the participants stated that they initiated their diagnostic process (58.3%). Medical professionals initiated diagnosis in 27.8% of cases.

> "Gynecologist concerned about osteopenia- referred to [general practitioner]. GP found Celiac with blood test and also low vitamin D." (P29)

> "It was noted that I was extremely anemic in 2005." (P66)

The remainder of diagnoses began with encouragement from friends/family (some whom were also diagnosed):

> "My weight dropped to 98 lbs and my mom demanded that I be seen by a gastro-specialist." (P8)

> "A friend strongly urged me to go to her naturopath to be check for gluten intolerance." (P14)

> "I self-diagnosed because my sister was diagnosed about 20 years before me and I began having classic symptoms (diarrhea)." (P16)

> "Had bad stomach pain- consulted sister who is gluten intolerant. Self-diagnosed by diet elimination." (P139A)

> "Friend heard of Celiac on NPR and suggested I get tested" (P150A)

Initiation of Diagnosis (Why)

Open-ended question: "Can you supply any other details about the context that started the diagnostic process?"

Approximately one-third of participants stated that the diagnostic process was started due to gastrointestinal problems (31.4%). Answers to this section were *highly* varied and also included: unexpected finds of colonoscopy (n=2), dermatitis herpetiformis (1), heartburn (1), osteopenia (1), joint pain (1), relative/friend diagnosed with 'gluten intolerance' (2), family member

diagnosed with Lupus Erythematosus (1), lack of positive response to years of psychiatric treatment (1), anemia (3), weight loss (2), migraines (2), constant sinus infections (1), and recurrent bouts of yeast infection (1).

> "I always had GI problems in high school, (doctors) told me it was IBS, got really sick- parasite in Mexico and came back and [doctors] tested me for Celiac- positive." (P1)
>
> "Suspected sensitivity to wheat based on systemic inflammation." (P16A)
>
> "Dermatologists discovered I had dermatitis herpetiformis." (P24)
>
> "Tried to determine what caused my heartburn." (P26)
>
> "I ended up in hospital." (P34)
>
> "Routine colonoscopy because of family history of colon cancer." (P40)
>
> "I went in because I just never felt good, always tired, muscles hurt, headaches almost every day." (P59)
>
> "Persistent rash. Consulted naturopath (once insurance finally covered). She diagnosed very low adrenal function and treated. Further testing showed hypothyroidism and then gluten intolerance. I ignored the [diagnosis] until eating several pieces of bread one morning resulted in joint inflammation (severe) within 30 minutes." (P58)
>
> "I was sick all the time, had bad skin, brittle hair and racked bleeding hands so I tried going to a naturopath." (P49A)

Interactions with Medical Professionals

Open ended question: How many and what kinds of medical professionals did you consult?

Please provide rough dates (or timing) for the steps in the process):

Number of medical professionals consulted

Participants most often saw 2 professionals leading up to diagnosis (mode = 2, n=12). Two participants did not consult professionals and were entirely self-diagnosed. It was not

uncommon for individuals to see multiple professionals along their path to diagnosis: 3 professionals (n=6), 4 (n=4), 5 (n=2). One saw 10 different professionals in pursuit of diagnosis.

> "GP- 1984, OB/GYN- 1995; gastroenterologist- 1996; endocrinologist 1996; naturopath 1998; GP (new) 2000, 2002; physical therapist 2006; allergist 2001, 2002, 2005)" (P112A)

Experiences with Medical Professionals

Some participants described negative experiences with various medical professionals and/or the diagnostic process:

> "Sister has celiac and lupus- changed doctor because she refused to test me when I asked." (P46, who later tested positive for 'classic' CD)

> "Chronic constipation- 30 years ago I was told to 'loosen up' because 'you have a spastic colon' by a very creepy doc. I didn't talk about this again until recently." (P66)

> "Dr. didn't agree to do (blood test) for Celiac. Do not think the Dr. was knowledgeable on gluten and those issues." (P143A)

Some participants noted positive experiences with medical professionals:

> "Under care of psychiatrists in 1987: Second doctor (neurologists and psychiatrist) after 6 years of management suggested investigating food because I responded well to anti-seizure medication. [The nutritionist] suggested trying ketogenic diet and/or modified Atkins- but had to get guidance from internist; Good nutritionist." (P59)

Testing

Six participants had genetic testing done, of which 5 indicated 'positive' results.

Blood testing was done for 22 individuals- 20 of which indicated 'positive' results. Three participants described their results as "off the chart" (P17, P19, P150A). Two individuals listed anemia under their blood test results (P48, P66).

Twenty individuals had endoscopies, of which 14 reported 'positive' results. Three participants were on a gluten free diet when endoscopies were done and had negative results (P17, P59, P61). Three participants mentioned the findings of precancerous polyps (P48, P61, P112A). P8 described their endoscopy results as "raging celiac".

Ten participants state additional testing including colonoscopies (P7, P34), MRI (P34), ultrasound (P34), CAT scan (P34), stool samples (P36, P61) and urinalysis (P34).

Diagnosis

Three participants indicated they were "gluten sensitive", 9 described themselves as "gluten intolerant", 20 were diagnosed with "Celiac Disease" and 2 had "gluten allergy".

Possible Diagnostic Confusion

Some checked multiple boxes for their diagnosis even through their listed test results indicated that they were CD- some of those who indicated that they were Celiac also indicated gluten intolerance (P36, P46, P48, P61). One participant indicated that she was diagnosed with 'wheat allergy' even though she reported daily long-term adverse reactions to food (P52). One participant stated that they were gluten intolerant and 'probably' Celiac because of their dermatitis herpetiformis (P61). One participant did not know their particular diagnosis at all: "Not clear during consultation- Dr. said stop eating gluten." (P7)

Family Diagnoses

Most participants were the first in their family to be diagnosed (n=27, 77.1%). Family members diagnosed first included sisters (n=4), brothers (n=2), granddaughter (n=1), and a female cousin (n=1). Some participants left additional comments about affected family members.

> "[My mother] died at 47 years of age, adrenal disorder, and highly suspect Celiac. [My father] died at 68 years old of Parkinson's. As I look back, I think he had Celiac too. My [siblings] will not be tested due to lack of reliable testing. They use elimination diets to identify foods that are causing illness. My eldest brother is very sick, with many diagnoses, which prevents him from tolerating the rigors of an elimination diet, and lacks health care." (P67)

> "My 3 yr old grand-daughter who is now 12 [was the first to be diagnosed]. Since that diagnosis we have 6 in the family, 4 generations ages 8-87 and probably more." (P17)

Time to Diagnosis

Twenty-three participants provided a timeline to diagnosis. Months to diagnosis varied greatly (median= 26 months, IQR= 3 -228 months, range 0-53 years). The most extreme delayed diagnoses were: 48 years to CD diagnosis (P71), 33 years to CD diagnosis (P67), and 28 years to 'gluten intolerant' (P68).

> "1990, gastroenterology, upper endoscopy for heart burn. Found possible Celiac but wasn't sure- said I didn't have usual symptoms- diarrhea, etc." (P26) (20 years to diagnosis, Celiac Disease).

> "Decades (early 1970s) of GI symptoms, various doctors, finally one that looked at celiac- 2006" (P36) (31 years to diagnosis, Celiac Disease).

> "I was having constant diarrhea, losing weight, severe stomach pains…Numerous trips to doctors from childhood on for stomach problems" (P71) (48 years to diagnosis, Celiac Disease).

> "My brother and I have been ill since childhood. Overtime symptom list expanded and symptoms worsened. Since 1978-2011: primary doctor, psychiatrist, women's doctor and surgeons, allergist, gastroenterologist (he said my stomach problems were

> emotional stress), naturopaths, eye doctor, dermatologist, cardiologist, neurologist." (P67) (33 years to diagnosis, Celiac Disease).
>
> Participant P68 was never formally diagnosed by medical professionals due to 'negative' blood testing. "[No diagnosis], but my doc suggested I try being gluten free for 3 months- which I did." This patient self-diagnosed as 'gluten intolerant'.

Average time to diagnosis for persons with "gluten intolerance" (211 ±116.83 months, n=5) was significantly longer than those who were diagnosed with CD (88.9 ±122.0 months, n=14) (Welch's $t=1.98$, $df=10.8$, $p<0.05$ single tailed). Given that both groups had extreme outliers, median values were also calculated: 240 months for 'gluten intolerant' and 36 months for CD.

SYMPTOMOLOGY

Proportions of persons reporting "none" (1) and "all of the time" for symptoms before diagnosis and after diet change are presented in Table 16.

Commonality

The most common symptoms reported prior to diagnosis related mostly to gastrointestinal issues. The mode for all symptoms prior to diagnosis was "1" or "none of the time" with the exception of abdominal distension (mode =3), abdominal pain (4), diarrhea (3), flatulence (3), and moodiness (bimodal= 1, 2). For these conditions 8.8%, 20%, 11.4% and 45.8% of participants were unaffected by these conditions prior to diagnosis (respectively). The most common symptom based on median was abdominal pain (median = "4" or "most of the time"). Abdominal distension, flatulence, diarrhea, chronic fatigue, frequent sinus congestion, anemia and moodiness all had a median of "3" or "some of the time". Esophageal reflux, constipation, brain fog, steatorrhea, bulky pale stool, frequent canker sores and failure to thrive had a median of "2" or "a little of the time".

Epilepsy and multiple sclerosis were not reported as symptoms within the study. PCOS was reported for 4 women. Two people reported symptoms of cerebellar ataxia prior to diagnosis. Two women reported problems with recurrent miscarriage at or before diagnosis. One person reported Crohn's Disease symptoms. Vitamin deficiencies were reported for 16 participants. Chronic depression was reported for 17 individuals. Numbness/pain in hands/feet was reported among 13 participants.

Some participants listed additional symptoms, although they did specify if there was change post-diet change: "Atypical bipolar, mitochondrial dysfunction, dementia, slowed speech, extreme chronic fatigue, short term memory loss, asthma, rosacea, and keratoconus" (P59); "Low thyroid, Low vitamin D levels" (P14); "Hyperactive thyroid" (P17); "Ridged fingernails, rosacea, facial acne" (P36); "Mucus in bowels" (P47).

Severity

Anemia and frequent sinus congestion were the most severe prior to diagnosis; 22.9% each of participants reported that these were problems 'all of the time'. Chronic fatigue, moodiness and abdominal distension ranked "5" among 20% of participants, each. Chronic esophageal reflux and diarrhea were reported "5" each for 17.1% of participants.

Symptoms Post-Gluten-Free Diet Change

Frequent sinus infections resolved entirely for 3 of 22 affected individuals and were otherwise reduced in all but 2 people upon diet change. Moodiness resolved entirely for 8 of 26 affected people, and was reduced in all but 2 persons. Esophageal reflux resolved for 1 person out of 19

affected and was reduced in the rest except for 1 person. Vitamin deficiencies resolved for 6 out of 16 affected individuals and were otherwise reduced in all but 1 person. Chronic depression resolved entirely for 5 of the 17 affected individuals and reduced in the remainder except for one. Twelve persons reported presence of itchy skin rash even after diet change. Numbness/pain in hands/feet resolved for 2 people and was otherwise reduced in all but one. Osteoporosis, osteopenia, and osteoarthritis did not respond to dietary change, except for in one individual. Symptoms of PCOS resolved entirely for 2 women, reduced for 1 and remained the same for one. One of the women that reported resolve symptoms also stated that she had surgery for the condition. After diet change only 1 still showed symptoms of cerebellar ataxia (out of 2). Recurrent miscarriage resolved for one woman (of 2) upon diet change. Only one person showed decreased symptoms for spinal cord problems (all of time to some of time). Atherosclerosis was reported for only one individual, it did not reduce in severity upon diet change. The person with Crohn's Disease symptoms reported a disappearance of symptoms after diet change. One person appears to have developed fatty liver disease after diet change.

For all symptoms considered, the percentage of persons reporting 'none of the time' increased after diet change ($p=1.18E-5$). The percentage of persons reporting chronic symptoms also decreased after diet change ($p=3.33E-7$).

	BEFORE DIAGNOSIS				AFTER DIET CHANGE			
SYMPTOM	Median	None (1)	Always (5)	No Answer	Median	None (1)	Always (5)	No Answer
abdominal distension	3	17.1%	20.0%	2.9%	2	40.0%	2.9%	5.7%
abdominal pain	4	8.8%	11.4%	2.9%	2	37.1%	0.0%	5.7%
anorexia	1	68.6%	2.9%	11.4%	1	88.6%	0.0%	11.4%
brain fog	2	40.0%	8.6%	2.9%	1	54.3%	0.0%	5.7%
bulky, sticky, pale stool	2	42.9%	2.9%	8.6%	1	48.6%	0.0%	5.7%
diarrhea	3	20.0%	17.1%	2.9%	2	45.7%	0.0%	5.7%
flatulence	3	11.4%	14.3%	2.9%	2	28.6%	0.0%	5.7%
failure to thrive	2	57.1%	14.3%	2.9%	1	82.9%	2.9%	5.7%
muscle wasting	1	57.1%	8.6%	5.7%	1	71.4%	0.0%	8.6%
steatorrhea	2	42.9%	8.6%	5.7%	1	71.4%	2.9%	8.6%
vomiting	1	85.7%	0.0%	2.9%	1	88.6%	0.0%	5.7%
weight loss	1	71.4%	8.6%	5.7%	1	80.0%	0.0%	5.7%
hair loss	1.5	48.6%	2.9%	2.9%	1	62.9%	0.0%	5.7%
anemia	3	40.0%	22.9%	2.9%	1	77.1%	2.9%	5.7%
frequent canker sores	2	45.7%	2.9%	2.9%	1	57.1%	0.0%	5.7%
osteoarthritis	1	65.7%	2.9%	2.9%	1	68.6%	11.4%	5.7%
rheumatoid arthritis	1	85.7%	0.0%	5.7%	1	88.6%	0.0%	8.6%
cerebellar ataxia	1	82.9%	0.0%	11.4%	1	82.9%	0.0%	14.3%
chronic fatigue	3	31.4%	20.0%	5.7%	1	48.6%	2.9%	5.7%
constipation	2	37.1%	14.3%	2.9%	2	45.7%	5.7%	5.7%
Crohn's disease	1	94.3%	0.0%	2.9%	1	94.3%	0.0%	5.7%
dental enamel defects	1	68.6%	8.6%	2.9%	1	62.9%	2.9%	20.0%
itchy skin rash	1.5	48.6%	11.4%	2.9%	1	60.0%	0.0%	5.7%
epilepsy	1	97.1%	0.0%	2.9%	1	94.3%	0.0%	5.7%
esophageal reflux	2	37.1%	17.1%	2.9%	2	40.0%	2.9%	5.7%
fatty liver disease	1	94.3%	0.0%	5.7%	1	91.4%	0.0%	5.7%
frequent miscarriages	1	85.7%	2.9%	8.6%	1	85.7%	0.0%	11.4%
spinal cord problems	1	88.6%	2.9%	2.9%	1	82.9%	2.9%	8.6%
obesity	1	71.4%	11.4%	5.7%	1	62.9%	5.7%	20.0%
osteoporosis	1	71.4%	11.4%	5.7%	1	77.1%	14.3%	8.6%
osteopenia	1	71.4%	17.1%	2.9%	1	95.7%	11.4%	11.4%
Multiple Sclerosis	1	94.3%	0.0%	5.7%	1	91.4%	0.0%	8.6%
numbness/pain in feet/hands	1	60.0%	5.7%	20.0%	1	62.9%	2.9%	5.7%
moodiness	3	45.8%	20.0%	2.9%	2	45.7%	0.0%	5.7%
chronic depression	1.5	48.6%	14.3%	2.9%	1	62.9%	2.9%	5.7%
Polycystic ovary Syndrome	1	77.1%	5.7%	11.4%	1	80.0%	2.9%	14.3%
frequent sinus congestion	3	34.3%	22.9%	2.9%	2	42.9%	5.7%	5.7%
high blood pressure	1	77.1%	11.4%	2.9%	1	74.3%	2.9%	5.7%
atherosclerosis	1	88.6%	2.9%	8.6%	1	82.9%	2.9%	14.3%

vitamin deficiencies	1.5	45.7%	11.4%	8.6%	1	62.9%	0.0%	14.3%

Table 16. A summary of reported symptoms and their severity for participants, "before diagnosis" and "after diet change." 1= "none", 2= "A little of the time", 3= "some of the time", 4- "most of the time", 5= "all of the time".

Qualitative Analysis

Two participants left additional comments regarding the effect of their diet change:

> "Anemia and other lab work connected to GF diet. Also osteopenia improved."
>
> "Changed diet immediately because I had felt so bad- worked miracles going off wheat/gluten items." (P143A)

Comorbidity

The results for comorbid conditions are summarized in Table 17 below and include approximate age at diagnosis for participants and number of affected family members per condition. Six participants did not fill out the comorbid conditions portion of the survey (for their own conditions). The most commonly reported conditions were: iron deficiency anemia (n=15), autoimmune thyroid disease (n=8), and constipation, depression and lactose intolerance (each n=8). Five participants each reported high blood pressure and inflammatory arthritis. Four persons each listed eczema, fibromyalgia, nervous stomach and irritable bowel syndrome. Iron deficiency anemia and autoimmune thyroid disease were diagnosed across wide age ranges: 12-60 years and 10-49 years, respectively.

Autoimmune thyroid disorders and high blood pressure were the two most common conditions listed for family members (13 each). Type-II diabetes was indicated for 12 family members total.

Condition	Affected Participants	Age at Diagnosis	Affected Family Members
Iron Deficiency Anemia	n= 15	12, 14, 15, 18, 20, 23, 25, 27, 30, 30, 40, 49, 50, 56, 60	n=7
Autoimmune Thyroid Disease	8	10, 30, 30, 33, 36, 40, 49	13
Constipation	7	?, 3, 15, 18, 20, 49, 50	9
Depression	7	?, 14, 20, 30, 35, 36, 50	10
lactose Intolerance	7	?, 30, 36, 37, 55, 56, 60	7
High Blood pressure	5	25, 30, 49, 59, 61	13
Inflammatory Arthritis	5	40, 40, 48, 50, 57	1
Eczema	4	0, 14, 18, 40	6
Fibromyalgia	4	21, 40, 40, 55	7
Irritable Bowel Syndrome	4	?, 30, 40, 50	9
Nervous Stomach	4	?, 7, 10, 42	7
Infertility (multiple miscarriages)	3	25, 25, 35	4
Addison Disease	2	49, 49	3
Raynaud's Disease	2	?, 51	4
Pernicious Anemia	2	45, 60	2
Rheumatoid Arthritis	2	?, 12	9
Autoimmune Hepatitis	1	10	3
Cerebellar Ataxia	1	61	1
Colon Cancer	1	53	8
Grave's Disease	1	30	0
Polycystic Ovary Disease	1	16	5
Primary Sclerosing Cholangitis	1	61	2
Psoriasis	1	40	3
Type II Diabetes	1	65	12
Vitiligo	1	20	2
Atrophic Gastritis	0	n/a	2
Behcet Disease	0	n/a	2
Crohn's Disease	0	n/a	4
Dermatomyositis	0	n/a	2
Hardening of the arteries	0	n/a	5
Lupus	0	n/a	4
Multiple Sclerosis	0	n/a	5
Myasthenia Gravis	0	n/a	2
Non-Hodgkins Lymphoma	0	n/a	2
Chronic Liver Disease	0	n/a	2
Seizures	0	n/a	4
Sjogren Disease	0	n/a	2
Small Intestinal Cancer	0	n/a	5
Type I Diabetes Mellitus	0	n/a	3

Table 17: Comorbid Conditions reported by participants. Approximate age at diagnosis is given where known for the participant. Number of affected family members is listed for each condition. Family includes mother, father, brother, sister, grandparents, children, and grandchildren.

DISCUSSION

Several factors that contribute to delayed diagnosis were identified within this study: confusion over terminology, issues with symptom classification, a need for further training of medical professionals and increased patient-doctor communication, and also low public awareness.

SAMPLE

The sample is a mostly older and well-educated group and clearly represents only a subset of individuals affected by gluten-averse conditions.

TERMINOLOGY AND CLASSIFICATION

Terminology and corresponding definitions direct both research and diagnosis of various conditions, creating and reinforcing the boundaries of 'disease'. Currently, medical research supports the existence of three gluten-averse conditions: gluten allergy, Celiac Disease, and non-Celiac gluten sensitivity. The division of delayed onset gluten-averse symptomology into CD and NCGS belies a much more complicated scenario, especially since CD clearly encompasses a broad spectrum of phenotypes. Continued grouping of CD into 'classic' and 'non-classic' (or any other binary system) will only hinder the research process of those who would seek to better understand such a complex disease and will continue to result in a decreased quality of life for those who are denied diagnoses based on outdated constructions of disease typology. As of 2011, NCGS stands to break from CD in to its own diagnostic category based on recent findings (Sapone et al 2011) and it is unclear how much of the 'non-classical' manifestations it will take with it. NCGS needs to be consistently recognized as a clinical entity by medical professionals. The very least of evidence for its existence should be in the presence of numerous support groups across the U.S. (and other countries) that have formed geared toward persons with "gluten intolerance". This 'third category' of gluten-averse reactions (NCGS) is clearly one

of the most misunderstood conditions- not merely for the lack of research into it as its own condition (described later), but also because the terms used to describe it are inaccurate and are also used to describe the other two gluten-averse conditions.

The impact of "typical CD" resulted in delayed diagnosis for at least one individual in the sample (P26), whose doctor found histological evidence for CD ("gold standard") but did not make the diagnosis due to lack of diarrhea, weight-loss, and other 'typical' symptoms.

CD Spectrum

Persons diagnosed with CD in this study showed a mix of phenotypes- mostly 'classic'. For example, P24 was diagnosed within the same year as 'classic' symptoms presented: abdominal distension, pain, steatorrhea, diarrhea, failure to thrive, and chronic fatigue. Blood testing showed elevated EMA and presence of anti-tTG. One was diagnosed with CD after an endoscopy/biopsy that was 'looking for something else' (P47). Their listed symptoms included esophageal reflux, canker sores, some constipation, and 'mucus in bowels'. No timeline to diagnosis could be provided for this person as they seem to exhibit "silent" CD.

NCGS as a Clinical Entity

Persons diagnosed with NCGS reported timelines to diagnosis that were more than 2 times as long as those for persons with CD. This is undoubtedly related to a general lack of clinical recognition of NCGS as a condition. Participant P68 eventually self-diagnosed after negative blood-test results and positive response to a gluten-free diet after 28 years of chronic constipation. Participant P34 was not diagnosed with 'gluten intolerance' for 16 years despite a previously diagnosed younger brother, and seeing both a naturopath and gastroenterologist since a hospitalization. (All blood tests came back negative and the participant did not have genetic markers for CD). Participant P112 visited 10 different medical

professionals over a period of 22 years leading up to diagnosis as 'gluten intolerant.' This patient also reported an *increase* in many symptoms post- diet change that might be indicative of a refractory state. One patient reported undergoing 3 colonoscopies, an ultrasound, MRI and various urine, blood, stool and saliva tests prior to visiting a naturopath for diagnosis with 'gluten intolerance' (P14).

The necessity of a paradigm shift in understanding CD (and NCGS) clinical manifestation (definition) cannot be understated. Any further categorization of symptomology in these (and other) conditions must be carefully constructed to avoid missed diagnoses. The lack of defined condition for NCGS has undoubtedly delayed or prevented diagnoses for many and limited the pursuit of research into NCGS as its own condition- much as the long-standing characterization of CD as a gastrointestinal disorder has delayed acknowledgement of the full CD spectrum.

Combatting the confusion over these conditions *must* start with the development of clear and consistent terminology, as well as the recognition of the full spectrum of conditions (including NCGS). Further misguided use of terms will only continue to delay the relevant diagnoses that need to be made for persons affected by these conditions. It should also be acknowledged that our labeling is subject to change upon new information. In the future, the CD spectrum may well split into definably separate conditions. However, it is this author's opinion that medical professionals and researchers seeking cut and dry etiologic explanations will not find it given the complexity of human physiology. A single disease phenotype may have many underlying developmental pathways and the medical research arena is only just beginning to appreciate the vast complexity of interactions between commensal bacteria and humans, which is just one of the likely contributors to gluten-averse conditions.

Continued Training for Medical Professionals

The diagnostic trajectories described by patients in this study indicate that many medical professionals *are* making CD diagnoses in good time frame: Patient P24 saw a dermatologist for skin condition that

was diagnoses as dermatitis herpetiformis. The patient was subsequently sent to a gastroenterologist for blood testing for CD. P29 saw a gynecologist who expressed concern about the patient's osteopenia and was referred back to their general practitioner. The GP performed both blood testing and endoscopy and diagnosed the patient with CD.

However, presentation of 'classic' CD did not lead to reliably faster diagnoses. It is possible that some of this delay might be accounted for by a lack of training/knowledge in previous decades and some results may be more indicative of improved training for doctors. For P26, an upper endoscopy in search for the cause of heartburn (in 1980) showed possible Celiac. Twenty years later a gastroenterologist performed a blood test and confirmed presence of auto-antibodies. One of the most delayed diagnoses was for a woman who had gastrointestinal symptoms since the 1970s and had visited many doctors. In 2006, a general practitioner checked for CD and found that she had genetic risk markers, and positive serology. Additionally, she had a younger brother who was diagnosed with CD at age 2.

Participant P1's delay in diagnosis however was the result of a *current* common misdiagnosis: "I always had GI problems in high school, told me it was IBS." This patient displayed all the common markers of 'classic' CD and also test positive for CD autoantibodies and presence of intestinal damage. P32 was sent to a gastroenterologist after constant diarrhea, bloating, and lots of gas. The gastroenterologist performed a colonoscopy but did not look for CD nor have any serological testing done. The patient subsequently saw an internist who ordered blood testing for CD, the results of which were positive.

Timeline to diagnosis was calculated based on *self-reported* initial presenting symptoms. If timeline to diagnosis took in to account the presence of common comorbid conditions, diagnosis length might be greatly increased for many patients in this study: P150A was diagnosed with iron deficiency anemia 9 years prior to diagnosis as "off the charts" CD. P143A also presented with iron deficiency, 3

years prior to CD diagnosis. Patient P70 was diagnosed with depression at age 20, eczema from ages 18-30, rheumatoid arthritis and had 4 miscarriages prior to diagnosis with CD at age 47. This would increase time to diagnosis from 2 to 27 years. Time to diagnosis for P61 could be extended to 36 years (from 20) if their reported diagnoses of eczema at age 14, iron deficiency anemia at 12, and nervous stomach at age 7 were physiologically related to CD. Of course the connections for these patients will never be verified. However, it should be taken as a reminder that highly variable symptomology, taken even without overt CD, should prompt medical professionals to rule out gluten-averse conditions. Furthermore, differentiation between CD and NCGS age at onset for different age groups is problematic because date of diagnosis cannot be reliably tied to actual onset.

Naturally, it is recommended that medical professionals continue to receive training per the most recent research on diagnostic procedure and interpretation of results.

Doctor-Patient Communication

Receptiveness to patient complaints and mutual participation in the diagnostic process are important in improving quality of life. The results of this survey indicate the following several doctor-patient communication items as opportunities for decreasing time to diagnosis.

Clarity of Diagnosis and Diagnostic Process

One patient was confused as to her exact diagnosis: “Not clear during consultation. Dr. said stop eating gluten” (P7). She also stated that her blood test showed ‘gluten intolerance’ and that her endoscopy/biopsy was negative. One might assume that the blood test looked anti-gliadin antibodies. Her diagnosis took place in 2009 when anti-gliadin tests had largely been abandoned in favor of more sensitive and specific anti-gliadin and anti-tTG. She identified herself as ‘gluten intolerant’ and ‘allergy to wheat’ though she may still qualify for diagnosis as part of the CD spectrum given her listed

symptoms: abdominal pain, diarrhea, constipation, itchy skin rash. Diagnoses should be made clear and associated complications explained thoroughly to aid the patient in taking steps toward initiation and maintenance of a gluten-free diet.

Participant P52 reports diagnosis with 'wheat allergy' and while indicating (prior to diagnosis) daily bouts of illness. The only improved symptom listed was flatulence. No testing was listed for this patient. Given that wheat allergy is an IgE-mediated immune response, continual ingestion would likely have resulted in extreme physical reactions. One participant indicated that they were 'probably Celiac' because of their dermatitis herpetiformis but definitely 'gluten intolerant' (P61). They are most likely 'definitely' Celiac since DH is the well-documented cutaneous version of CD.

Seriousness of Condition

Approximately 40-85% of CD persons stick to the strict gluten free diet required by diagnosis (Ciacci et al 2002; Hogberg et al 2003). The fact that complications of CD are at least partly correlated with poor diet adherence means that doctors need to stress the importance of diet change in the treatment of the condition. Diagnosis with CD, NCGS, or GA need to be taken seriously by those affected. Persons affected by GA are much less likely to ingest gluten except on accident due to the immediate physical reaction. Delayed onset conditions should be taken just as seriously. The first step in emphasizing the seriousness of the condition is clarity of diagnosis.

Self-Diagnosis

Some patients pursue diagnosis entirely on their own such as P16A, who self-diagnosed as 'gluten sensitive' based on "personal reading". Such situations highlight several things: (1) *Clear, consistent and accurate information* for these conditions must to be available to the public. (2) Some persons may be reluctant to trust medical professionals with help in diagnosis.

Testing Family Members

The diagnosis of a family member should prompt a quicker screening and diagnosis for relatives than what is observed in this sample. In some cases there was a lengthy delay in the diagnosis of the participant after a family member had been diagnosed. Others reported that family members were reluctant to pursue testing for one reason or another. Given the genetic associations of this condition, diagnosis of a family member *should* spur testing for other family members.

Cases exist where a person is diagnosed and there is a subsequent string of diagnoses in the family, as for P17, (6 family members of four generations). Whether this was medical or family initiative is not provided by the respondent. In other cases, there is a large time lapse in diagnoses between family members. It is possible that these individuals may have had an increased quality of life if they had been diagnosed shortly after their family member:

> "I self-diagnosed because my sister was diagnosed 20 years before me and I began having classic symptoms" (P16). (Remember that some of the classic symptoms are associated with destruction of the small intestine, a step that is not required for diagnosis in the CD spectrum).

> Patient P34 "ended up in the hospital" before getting diagnosed as 'gluten intolerant'. Her diagnosis was delayed by approximately 16 years despite having a younger brother diagnosed prior.

> P36, was diagnosed as CD after approximately 31 years at age 65 (positive blood test and histology). Her brother was diagnosed at age 2. Her current symptom list before and after diet change may indicate a refractory state: not a single item is listed as improved and some of them appear to increase post-diet change.

In one case, a patient reports that when her sister was diagnosed with CD, she requested the doctor to test her as well and was denied (P42).

Open Dialogue

Currently, clinical diagnosis by general practitioners and many other specialists is exclusively focused on laboratory testing and medical interpretation of results. The spectrum of gluten-averse conditions has a broad array of symptomology and a 'big picture' view of patient symptomology may assist in diagnosis. Consequently, open discussion between medical professionals and patients should be encouraged. Current understanding of the CD spectrum strongly suggests that a continued reliance on serological and endoscopic tests alone will fail to catch numerous phenotypes. Furthermore, given that the best current model for pathogenesis involves 'multiple hits' and that environmental triggers may vary between individuals, it could be recommended that clinicians take in to account things such as NSAID consumption, recent or repeated bacterial infections, and stress when determining if a patient might benefit from a gluten free diet. To this end, open dialogue should be encouraged.

Increased Quality of Life and Avoiding Complications

Delayed diagnosis is significantly correlated with increased risk for complications and development of refractory conditions in CD. The need for quicker diagnoses is further underwritten by the work of Biagi and Corazza (2010) who suggest that the differences in mortality between populations with similar CD prevalence may relate to gluten consumption levels prior to diagnosis. Persons with gluten-averse conditions need to be diagnosed as quickly as possible to experience the increased quality in life associated with diet change.

Increased Public Awareness

Given the delay in onset and associated symptomology, it is likely that many individuals (1) either do not suspect diet a factor in various illness (however mild) and/or (2) see certain 'mild' ailments (such as headache, depression, etcetera) as normal and not deserving of medical attention (and corresponding diet change). The sample described here represents a group who is very proactive about their own health. Diagnoses to NCGS were markedly longer than that for CD, which is understandable given that research evidence for NCGS as its own condition surfaced just this year. The patients here who continually pursued NCGS diagnosis may not be typical of the population in general. Thus, one could easily speculate a sizable number of the US population suffering needlessly from various avoidable symptoms. Note that 1% of the United States population alone is equal to approximately 3 million people.

The need for accurate information available to the public must also be emphasized. Misinformation about gluten-averse conditions will only continue to self-perpetuate and delay diagnoses.

Possible Mass-Screening

CD has been described as a 'hidden epidemic' (Green and Jones 2006) and fulfills recommended criteria of the World Health Organization (WHO) for mass screening (1967): CD is an important health problem for which there is a reliable treatment (gluten free diet). Medical facilities are available for mass screening. A latent stage of the disease has been shown to exist and the natural history of CD is fairly well understood (especially relative to other autoimmune conditions). The cost of early diagnosis and treatment is cheaper than the treatment of the long term health effects that result from continued symptomology, development of comorbid conditions and disease complications. Green et al (2008) tracked cost of care for several cohorts over a period of 5 years: A group with newly diagnosed CD, and

one group each of persons without diagnosis but who displayed 1, 2 or 3 CD-associated symptoms/conditions. Relative value based medical costs (RVU) were calculated for the four groups and it was found that "direct standardized medical costs" were 24—33% lower for the CD relative to Control group 1, 24-38% lower than compared with control group 2, and 31-38% lower than those for control group 3 ($p < 0.05$, $p < 0.05$, $p < 0.01$, respectively). Cost discrepancy is attributed to less spent on office visits, laboratory work, as well as various diagnostic, imaging and endoscopy/biopsy procedures.

A large multicenter study of CD by Fasano et al (2003) noted that physicians and/or insurance companies would not pay for intestinal biopsies in approximately 21% of patients who had positive EMA serology. The justification was that the costs of the procedure were not warranted given the symptoms. Such a situation underscores a gross widespread misunderstanding of the serious condition and also hinders diagnostic procedures for patients who rely on insurance, in cases where doctors are willing to do an endoscopy. In such cases, doctors are limited by the insurance coverage of their patient. Current average national cost for an endoscopy and biopsy is approximately $2700 (New Health Choice 2010).

Minimally, more routine blood testing for food allergy and auto-antigens is recommended for family members of persons with gluten-averse symptoms and perhaps all who exhibit any of the autoimmune disorders that are typically over represented in the families of CD patients.

CONCLUSIONS

Delayed diagnoses in the spectrum of gluten-averse conditions may be reduced through the following: (1) Standardization of terminology and definitions of CD, NCGS, and GA, with acknowledged tentativeness that some of these labels may be revised as new research becomes available. Suggestions for standardization have been put forth here. (2) Continued and more widespread acceptance of CD as a spectrum for which previous diagnostic standards are inadequate. Negative serology and endoscopies should not halt diagnosis toward gluten-averse conditions. (3) Recognition of NCGS as a clinical entity. (4) Continued training for medical professionals in recognizing and diagnosing these conditions. (5) Improvements in doctor-patient communication with special attention to clarity of diagnosis, involvement of the patient in the diagnostic process, emphasis on the seriousness of the condition and need for strict gluten-free diet adherence. (6) Increase in frequency of testing for family members and other high risk populations (such as persons with autoimmune disorders). (7) An increase in public awareness and appreciation of these conditions are medical entities; reliable and accurate publicly available information for gluten-averse conditions. (8) Consideration of mass screening, which has been effective in several European countries.

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