CYREX® Laboratories

INTERPRETIVE GUIDE

ALZHEIMER'S LINX™

ALZHEIMER'S-ASSOCIATED IMMUNE REACTIVITY

THANK YOU

FOR CONSIDERING CYREX LABS.

We are excited to introduce Alzheimer's LINX™, the new, ground-breaking test panel for detecting Alzheimer's-Associated Immune Reactivity.

Alzheimer's LINX™ is the result of years of research in environmental factors that are known to have an association with the development of Alzheimer's disease.

Many immune disorders have underlying immune reactivity that can simmer for years before the associated disease forms. These underlying immune reactivities, in the form of antibodies, can be detected in the blood, sometimes 20 or more years before the diagnosis of the disease. They can be detected against a variety of tissues and environmental factors.

Antibodies against environmental triggers, such as pathogens, toxic chemicals and food antigens, have the potential for cross-reacting with human tissue, resulting in autoimmune reactivity.

The biomarkers in Alzheimer's LINX™ can shed light on a normally unseen perspective of the immune system's potential role in the development of Alzheimer's disease.

The Cyrex Team

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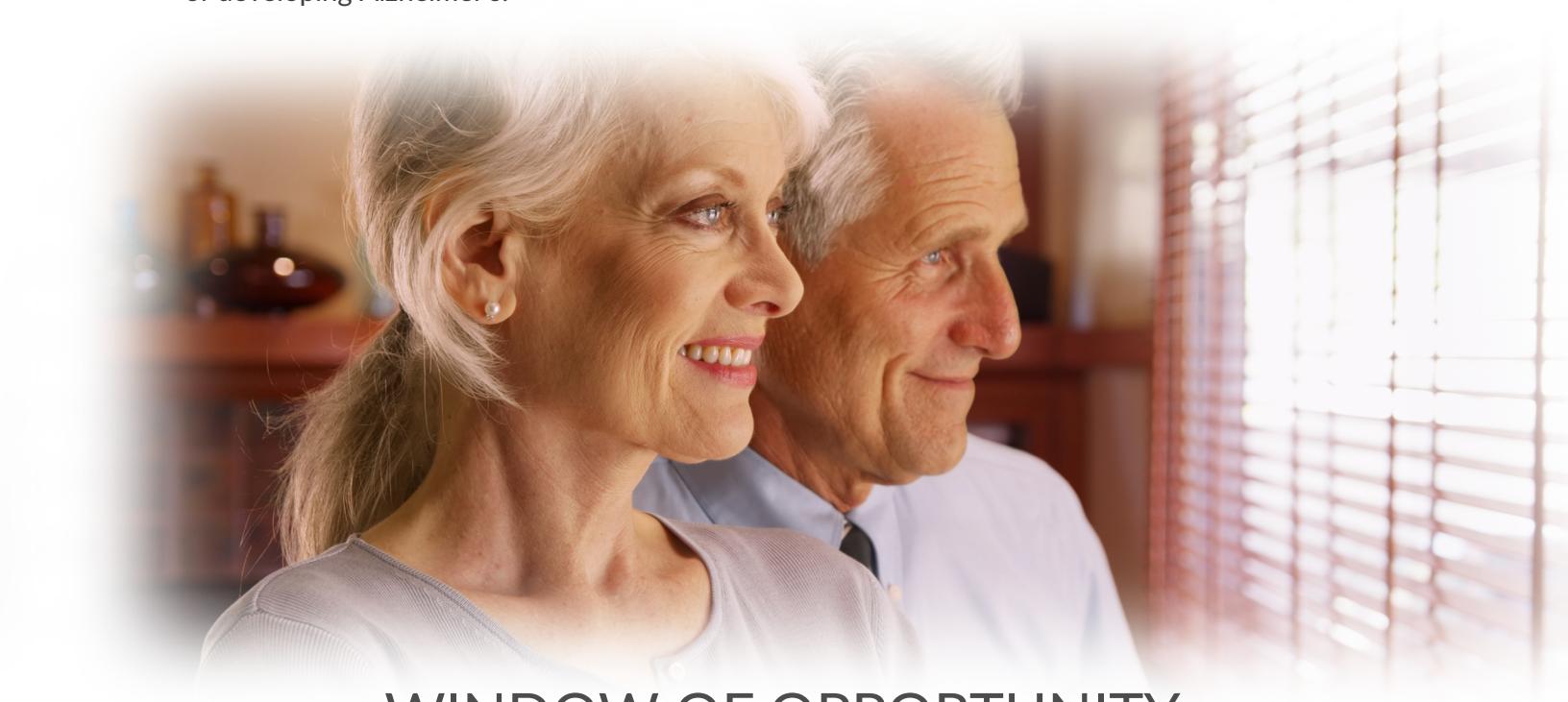
WELCOME TO A NEW FRONTIER IN UNDERSTANDING ALZHEIMER'S

For decades the prevailing belief has been that genetics is the cause of Alzheimer's disease. In the last few years, new research is indicating that Environmental Factors such as certain pathogens, chemicals, and foods as well as certain internal factors which, in combination, can increase the risk of developing Alzheimer's.



WINDOW OF OPPORTUNITY FOR INTERVENTION

Cyrex's Alzheimer's LINX™ test panel assesses seven key groups of antibodies produced by the immune system against internal factors as well as certain environmental triggers (pathogens, foods, and toxic chemicals) that are linked to the potential development of Alzheimer's disease.

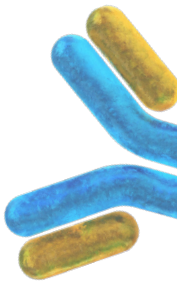
At the very early stage of autoimmune reactivities, these detected antibodies may not yet be disease-forming. However, if these antibodies attack the targeted tissue, on a chronic basis, they may contribute to the progression of many autoimmune diseases, including Alzheimer's disease.

It can take many years from the time elevated levels of these antibodies are first detectable in the blood, to the full disease onset. Antibodies have the potential to become disease-specific if they cross-react with particular tissue antigens involved in different autoimmune diseases. If the environmental triggers are removed, the reduction of their related antibodies in the blood may minimize damage to certain tissues, linked to the development of Alzheimer's disease.

DETECT antibodies

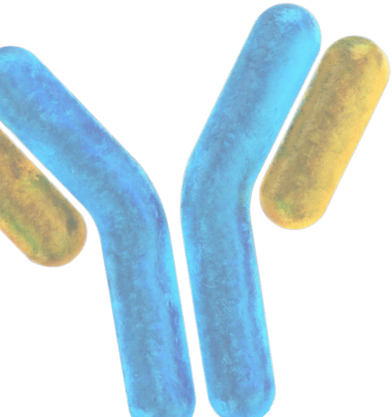
20 YEARS

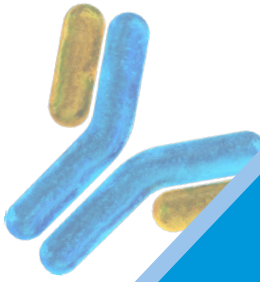
BEFORE symptoms actually appear...



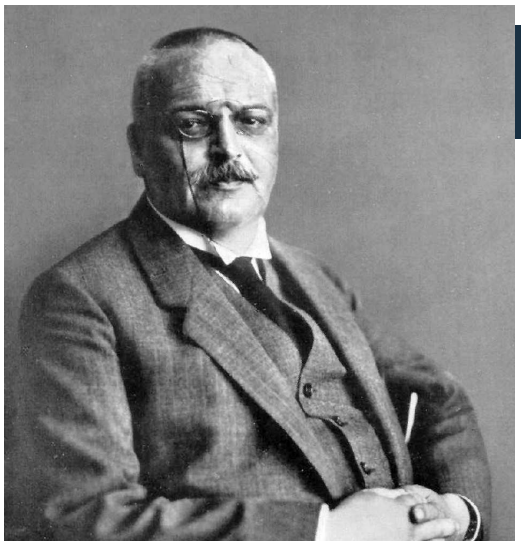
"...the power of Predictive Antibodies"

Dr. Aristo Vojdani, Ph.D, M.Sc., C.L.S.





OVERVIEW



Dr. Alois Alzheimer

The association between old age and increasing risk of dementia was first made by ancient Greek and Roman philosophers and physicians.

In 1901, German psychiatrist Alois Alzheimer identified the first case of what became known as Alzheimer's disease (AD), named after him, in the case of a fifty-year-old woman he called Auguste D. He followed her case until she died in 1906.

Here are some facts about Alzheimer's disease.

- Alzheimer's disease is the most common form of age-related dementia
- Alzheimer's disease is characterized by cognitive impairment as a result of:
  - » Inflammatory immune reaction in the brain
  - » Neuron entanglement (neurofibrillary tangles)
  - » Amyloid-Beta deposition or plaque formation in the brain
  - » Neurodegeneration
- Alzheimer's disease is one of the biggest medical challenges of our time
- 5.7 million people in the USA and more than 47 million people worldwide live with AD, and by 2050 this number may exceed 120 million if no effective prevention strategies are found, (Alzheimer's Association 2018)
- Substantial evidence suggests that leading a healthy lifestyle, that includes regular exercise, lowers the risk of developing AD by enhancing the production of brain derived neurotrophic factor (BDNF)

ALZHEIMER'S DISEASE AND ENVIRONMENTAL FACTORS

The latest research shows that environmental triggers play a far more influential role in the development of AD than previously thought. Over 95% of AD cases are due to **environmental factors**, rather than genetics. Scientists have shown that the development of AD is based on ongoing inflammation, microglial activation, and the buildup of two proteins in the brain called **amyloid-beta** and **tau**.

Amyloid-beta acts like the antibiotic for the brain to clear inflammation from various causes. Unfortunately, if inflammation becomes chronic, the amyloid-beta peptides become sticky, form clumps and attach to neurons where they can continue to grow and ultimately kill the neurons. Tau proteins are activated by the body in response to clear them away.

In patients with AD, the presence of amyloid-beta aggregates promotes the abnormal phosphorylation of tau protein, which blocks the clearance of amyloid-beta clumps, as well as the formation of neurofibrillary tangles within neurons.

Together, this abnormal accumulation of amyloid-beta plaques and neurofibrillary tangles may cause neuronal and synaptic loss in the brain, which, over time, results in neurodegeneration.

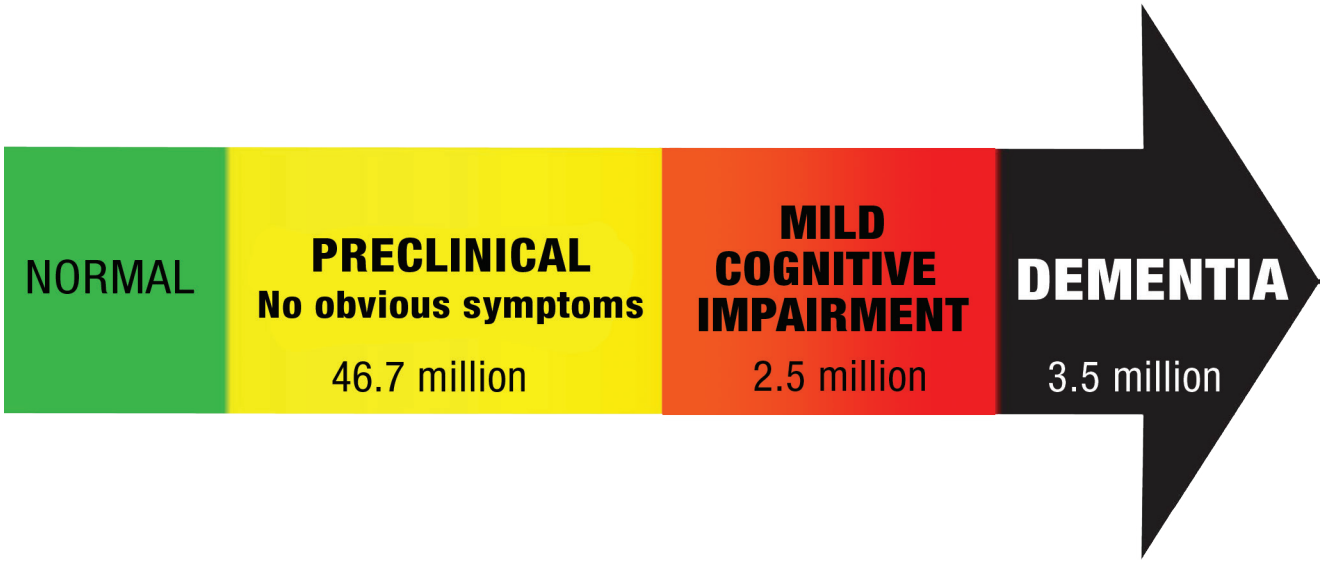
One of the hallmarks of AD is the abnormal accumulation of amyloid-beta due to inadequate clearance by tau protein.

The role of environmental antigens, related antibodies, and cross reactivity with brain tissues are presenting new areas to look at for the sources of the inflammation, leading to AD.

The pathological changes of AD are thought to begin up to 20 or more years before any clinical symptoms may appear. According to Brookmeyer *et al.*, 46.7 million Americans were in one of the preclinical stages of AD in 2017. Lifestyle and environmental factors play a significant role in the development of late-onset AD.

These changes from normal are classified into three major stages:

- First stage: Preclinical AD
- Second stage: Mild cognitive impairment
- Third stage: Dementia because of AD



As a central component of future patient care, this understanding of the role of chronic inflammation in the pathological changes in AD highlights the importance of early detection and identification of the environmental factors that contribute to the progression of the disease.

THE KEY PROCESSES OF NEURODEGENERATION

Knowledge of the development of preclinical Alzheimer's disease is important because persons may be more likely to benefit from disease modifying treatments if interventions occur before the occurrence of significant brain damage.

Environmental factors affect physiology to the point of neurodegeneration through three pathways. Understanding these pathways plays a critical role in the creation of a plan of action for patients with positive test results.

1. **The gut-brain pathway:**

    a. Exposure to environmental factors/antigens (food proteins, chemicals, pathogens and an unhealthy, stressful lifestyle)

    b. Disturbance in the gut microbiome and/or gut barrier breach (environmental factors/antigens enter the bloodstream), leading to immune reactivity and systemic inflammation as the immune system reacts to the environmental factors causing inflammation

    c. BBB breach (entry of inflammatory molecules into the brain), resulting in the entry of environmental antigens and cross-reactive antibodies into the brain

    d. Neurodegeneration leading to disease (the antibodies and environmental antigens damage neuronal tissues in the brain; this worsens with time)

2. **Oral/nasal pathway:**

    a. Exposure to oral and nasal antigens (pathogens or chemicals/heavy metals); migration of these antigens to the brain through the tonsils, nasal passage, and olfactory sensory axons

    b. BBB breakdown, resulting in the entry of environmental antigens and cross-reactive antibodies into the brain

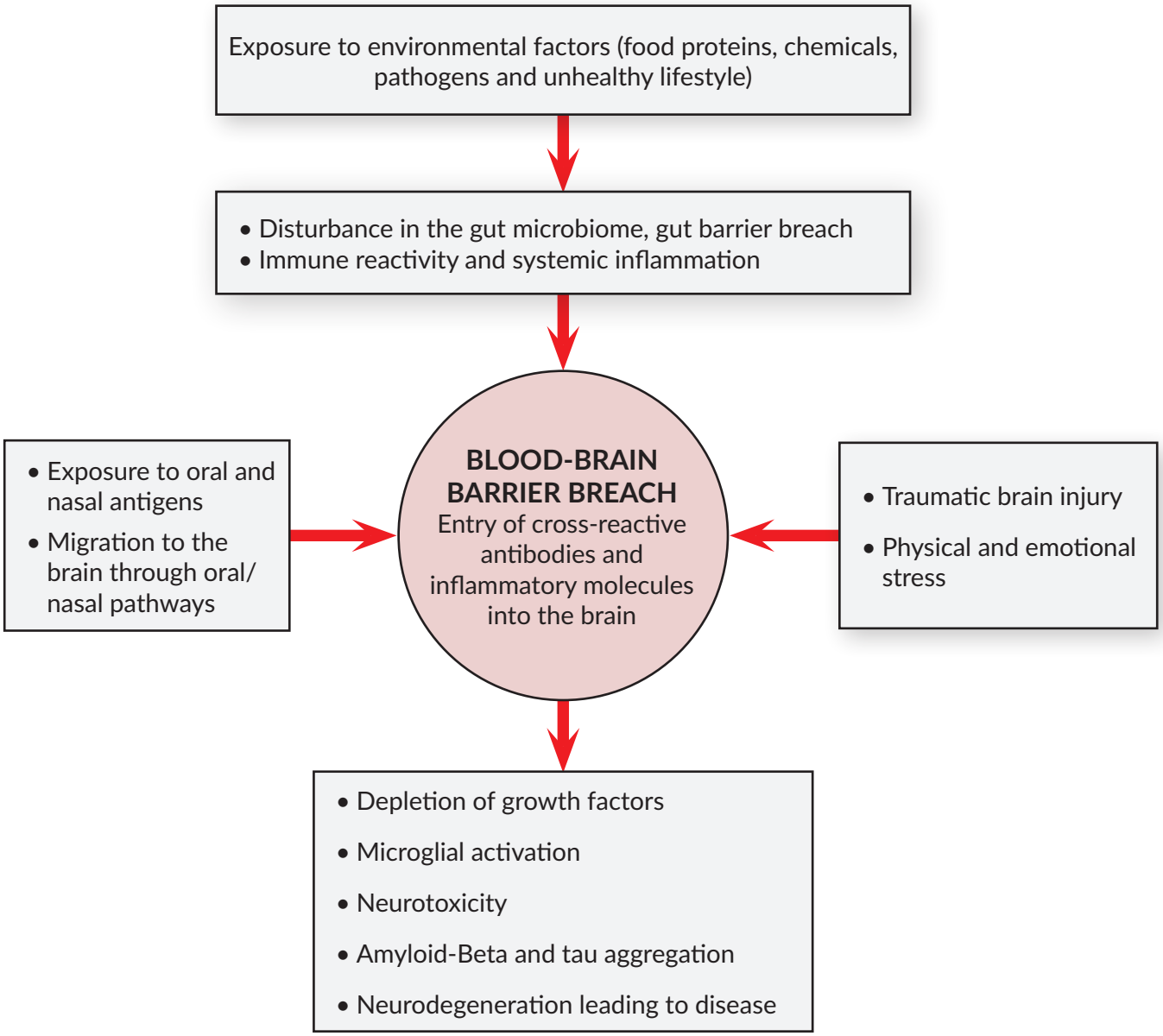
    c. Neurodegeneration leading to disease

3. **Traumatic Brain Injury (TBI) pathway:**

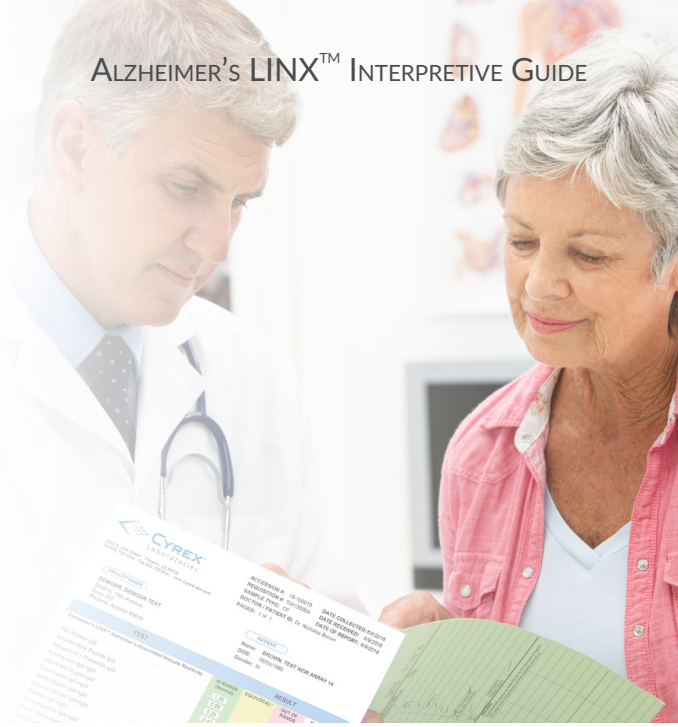
   a. TBI;

   b. Physical and emotional stress from TBI breaks the BBB, resulting in the entry of environmental antigens and cross-reactive antibodies into the brain

   c. Neurodegeneration leading to disease



HOW TO READ THE REPORT



Using the gold standard enzyme-linked immunosorbent assay (ELISA) methodology, **Cyrex's Alzheimer's LINX™** measures IgG antibodies against 39 endogenous (internal) and exogenous (environmental) antigens presented in seven key groups.

ENDOGENOUS FACTORS: brain proteins; brain growth factors; blood-brain barrier (BBB) proteins; and, enteric nerves, enzymes, and neuropeptides.

EXOGENOUS FACTORS:
pathogens, chemicals, and food antigens that cross-react with the amyloid-beta peptide.

Results are reported by the ELISA index and are classified as "in range" (negative), "equivocal" (low positive), and "out of range" (positive). The higher the ELISA index, the higher the level of antibody against the specific tested antigen.

TEST	RESULT			
Alzheimer's LINX™ Alzheimer's-Associated Immune Reactivity	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Tau Protein IgG	1.35			0.2-1.7
Amyloid-Beta Peptide IgG	0.95			0.2-1.8
Rabaptin-5 + Presenilin IgG	0.25			0.2-2.0

Reference ranges were developed using blood samples from 168 donors (more than four times the minimum requirement by the federal agency, Clinical Laboratory Improvement Amendments (CLIA), which sets standards and issues certificates for clinical laboratory testing in the USA). After analyzing these specimens, their mean ELISA index values were calculated. Any sample with an ELISA index number that falls below one standard deviation (SD) above the mean (mean+1SD) is considered "in range," an index between mean+1SD and mean+2SD is considered "equivocal," and any ELISA indices over mean+2SD are considered "out of range."

About 5% of the population suffers from antibody deficiency. Low total immunoglobulins may result in low antibody indices. As a result, Cyrex offers a total IgG, IgA and IgM assay (GAM), as an add-on test, at no additional charge, to help practitioners more accurately interpret test results. All antibody test results should be interpreted in the context of total IgG, IgA or IgM.

THE SEVEN KEY TEST GROUPS

1. Brain Proteins
2. Growth Factors
3. Enteric Nerve, Enzymes and Neurological Peptides
4. Pathogens
5. Chemicals
6. Foods Cross-Reactive to Amyloid Beta
7. Blood-Brain Barrier and Neurofilaments

There are at least 7 important areas in the pathophysiology of Alzheimer's disease (AD) that are also useful for the early detection of contributing factors and the management of this disorder.

1. BRAIN PROTEINS

These self-tissue proteins have been pinpointed as being key in the development of AD, and can potentially be the target of autoantibodies detectable in the blood:

- Tau Protein
- Amyloid-Beta Peptide
- Rabaptin-5 + Presenilin
- Alpha-Synuclein

The immune system makes antibodies to the brain proteins due to various reasons, which occur primarily outside the brain. These antibodies circulate in the blood and are not a threat to the brain as long as the blood-brain barrier is intact. However, if the BBB is compromised, these antibodies can penetrate the brain, react with brain proteins and result in neuroinflammation and neurodegeneration.

Elevated antibody levels against one or more of these amyloidogenic (plaque-forming) proteins puts a person at a higher risk for developing AD.

TAU PROTEIN

Interpretation of IgG antibody elevation against Tau Protein

Tau Protein is a microtubule-associated protein that is primarily found in the neurons of the central nervous system. Its job is to keep the neurons naturally free of amyloid-beta clumps and other toxic forms of proteins. Due to oxidative stress in patients with cognitive decline, tau becomes phosphorylated, misfolded, or aggregated.

This change in the molecular structure of tau protein makes it a target of the immune system, which can result in the production of anti-tau antibodies.

High levels of IgG antibodies against phosphorylated tau are detected in the blood of patients with mild cognitive decline and in patients with AD

Low amounts may be detected in elderly individuals without cognitive decline, which may be considered due to the natural aging process and accompanying destruction of neurons.

AMYLOID-BETA PEPTIDE

Interpretation of IgG antibody elevation against Amyloid-Beta Peptide

Amyloid-Beta Peptide is a key peptide that is involved in the pathology of AD. It is released by the amyloid precursor protein and acts like an anti-microbial peptide.

The detection of high levels of IgG antibodies against amyloid-beta peptide usually indicate the direct involvement of amyloid-beta peptide in the production of these antibodies.

Another possibility is that antibodies are produced against antigens of various foods and pathogens which share structural similarity or cross-react with Amyloid-Beta.

Furthermore, IgG antibodies against amyloid-beta peptide may be produced due to exposure to toxic chemicals that form new antigens, which resemble misfolded amyloid-beta peptide. If these antibodies cross the BBB, due to their reactivity with the amyloid-beta peptide, they may promote aggregation that contributes to the destruction of neurons.

Until recently, it was thought antibody to amyloid beta could be protective or pathogenic. In either case, the body is reacting to amyloid beta, whether it originated from the brain or the gut. Binding of antibody to amyloid beta is going to neutralize its defense function and is going to allow spread of pathogens in the brain as well as the rest of the body.

RABAPTIN-5 + PRESENILIN

Interpretation of IgG antibody elevation against Rabaptin-5 + Presenilin

Rabaptin-5 protein, a relatively recent discovery, is located in the microvascular endothelial cells and is involved in the regulation of neurotransmitter release and neuronal growth.

The detection of high levels of antibodies against rabaptin-5 can indicate a breach in the BBB due to environmental triggers and molecules that originate from the gut, which cause inflammation.

These antibodies also interfere with neurotransmitter release and the regeneration of damaged neurons.

Furthermore, due to cross-reactivity between rabaptin-5 and neurocrescin, a very important growth factor, the production of antibodies against rabaptin-5 may deprive neurons of this important growth factor, which is necessary for neuron reproduction and growth.

This process contributes to neurodegeneration.

Presenilin is the subcomponent protein of gamma-secretase that is responsible for the essential conversion of amyloid precursor protein into amyloid-beta peptides. Presenilin is prone to a mutation that enhances the production of toxic amyloid beta 42, leading to excessive aggregation of this peptide. Antibodies against presenilin and its cross-reactive epitopes that originate from pathogens or food antigens can cross the BBB. It may also contribute to the accumulation or aggregation of the toxic peptide amyloid beta 42 and the induction of amyloid plaque formation.

ALPHA-SYNUCLEIN

Interpretation of IgG antibody elevation against Alpha-Synuclein

Alpha-Synuclein is a protein found abundantly in brain neurons.

Detection of high levels of IgG antibodies against alpha-synuclein may indicate that factors such as pathogens and their toxins (lipopolysaccharide, cytolethal distending toxins), toxic chemicals, and even food antigen antibodies have managed to breach the BBB.

After penetrating the BBB and binding to the neurons, these invaders can cause aggregation of of alpha-synuclein into Lewy bodies, which could be followed by the induction of neuronal cell death, resulting in antibody production against alpha-synuclein and cellular components.

High IgG anti-synuclein antibodies are detected in patients with AD, inherited forms of Parkinson's disease (PD) and other neurodegenerative disorders. Ninety percent of patients with familial (genetic) forms of PD have been found positive for autoantibodies against alpha-synuclein, but this is not as common in the sporadic form. Lewy bodies have been found in intestinal enteric nerves, leading to the hypothesis that the intestine might be an early site of PD in response to environmental toxins, undigested food molecules, or pathogens. The detection of alpha-synuclein antibody in the blood should therefore also be interpreted in the context of immune reactivity to enteric neurons, and components of the intestinal and brain barriers.

2. GROWTH FACTORS

Growth factors help brain cells to grow and heal, but they don't function well in AD. The detection of antibodies against them in the blood may be a sign of trouble.

- Beta Nerve Growth Factor
- Brain-Derived Neurotrophic Factor
- Neurotrophins
- Somatotropin

Elevated antibody levels against one or more of these nerve growth factors puts a person potentially at a higher risk for developing AD.

NERVE GROWTH FACTORS

Interpretation of IgG antibody elevation against Nerve Growth Factors

As the name implies, Nerve Growth Factors are crucial for nerve cell growth, differentiation, and neuronal cell regeneration. Neurotrophins, are a family of proteins belonging to a class of growth factors. Somatotropin is a growth hormone secreted by the pituitary gland.

The detection of high levels of antibodies against beta nerve growth factor, brain-derived neurotrophic factor, neurotrophins, and somatotropin, and the specific binding of their antibodies, may indicate their inactivation, loss of function, or compromised function.

Imagine what would happen to your garden without the addition of fertilizer to the soil for 20 or more years, or if you put a tarpaulin on the soil and then added fertilizer. Growth-factor-specific antibodies would act like the tarp on the soil, preventing the fertilizer from getting to the roots of the plants; after enough time has passed, the plants would die.

Moreover, anti-amyloid-beta peptide has been shown to cross-react with all these growth factors; if IgG antibodies against these growth factors are present in the blood and cross the BBB, these antibodies can bind to amyloid-beta monomers, converting them to clumps and eventually into plaques. Therefore, the presence of antibodies to growth factors in the blood may turn into a double whammy: 1) depriving the brain of very important nutrients; and 2) binding to amyloid-beta peptide, and together, causing degeneration of neurons.

3. ENTERIC NERVE, ENZYMES AND NEUROLOGICAL PEPTIDES

The structural similarity between the central and enteric nervous systems suggests that an antibody-induced disease process affecting the central nervous system could also involve its enteric counterpart.

- Enteric Nerve
- Vasoactive Intestinal Peptide (VIP)
- Transglutaminases

Similar to events that may occur in the central nervous systems, elevated antibody levels against one or more of these components (enteric nerves, VIP, or transglutaminase proteins) can also put a person potentially at higher risk for developing AD.

ENTERIC NERVE

Interpretation of IgG antibody elevation against Enteric Nerve + Vasoactive Intestinal Peptide (VIP)

The enteric neurons (ENs) represent a vast neurological network that is organized into major ganglia. The ENs are distributed throughout the entire gastrointestinal tract and extend out to the biliary tract and pancreas. ENs are involved in transmitting sensory information between the enteric nervous system (ENS) and the central nervous system (CNS). The close structural similarity between the ENS and CNS suggests that an antibody-induced disease process affecting the central nervous system could also involve its enteric counterpart.

The presence of high levels of IgG antibodies against components of the Enteric Nervous System (ENS) may interfere with the functioning and communication of this important network with the immune system and the brain. Therefore, elevated levels of IgG antibodies against ENS components may also affect the CNS. Because of the interconnection of the gut, brain and immune system, high levels of IgG antibodies against ENS components could interfere not only with bowel movement and transmucosal fluid exchange in the small and large intestines, but also with the function of the immune system and the brain.

In fact, high levels of antibodies to ENS tissues are detected in patients with multiple sclerosis (MS) and other autoimmune diseases. It is thought that autoimmunity against ENS components may be the cause of intestinal disorders seen in patients with MS, who simultaneously exhibit IgG antibodies against key neural antigens including myelin basic protein, myelin oligodendrocyte glycoprotein, and proteolipid proteins.

In the case of AD, it has been shown that antibodies against amyloid beta react strongly with the enteric nerve. This indicates that production of antibodies against the enteric nerve would affect amyloid beta, and conversely antibodies that are produced against amyloid beta can affect enteric nerve function. Furthermore, if high levels of IgG antibodies against ENS cross the BBB, their tendency to bind to amyloid-beta peptides can contribute to clumping and plaque formation.

VASOACTIVE INTESTINAL PEPTIDE (VIP)

Interpretation of IgG antibody elevation against Enteric Nerve + Vasoactive Intestinal Peptide (VIP)

Vasoactive intestinal peptide (VIP) is a polypeptide that plays a central role in regulating the immune and nervous system functions, not just in the gut, but also in many organs/tissues including the heart, lungs, thyroid gland, kidney, urinary tract, and genital organs.

Thus, the detection of high levels of IgG antibody against VIP may indicate interference with the functionality of this polypeptide and a variety of tissues, including the gut. Furthermore, because VIP is a widely distributed neuropeptide in both the central and peripheral nervous systems, the production of antibody against VIP may suppress the anti-inflammatory and regulatory functions of VIP in the gut and the brain.

Scientists have shown that antibodies against amyloid-beta peptide react strongly with VIP antibodies. Therefore, the passage across the BBB of elevated levels of IgG antibodies produced against VIP may lead to the binding of IgG anti-VIP to amyloid-beta peptide, contributing first to amyloid-beta aggregation, and then to the destruction of neurons.

TRANSGLUTAMINASES

Interpretation of IgG antibody elevation against Transglutaminases

Transglutaminase 3, 6, and **microbial transglutaminase:** Transglutaminases are a family of enzymes that form protein polymers, like scaffolding, which are vital in the formation of barriers and stabilizing structures. Tissue Transglutaminse-3 (tTG3) is present mainly in the epidermis, and to a lesser extent in the placenta and the brain. Tissue Transglutaminse-6 (tTG6) is present in neural tissue.

In this interpretative guide, we put a significant emphasis on the role of microbial transglutaminase (mTG) used to make meat glue which is widely used as a food additive.

Because it has been shown that microbial transglutaminase cross-reacts with human tissue transglutaminase in the gut, hair follicles and in the brain, detection of high levels of IgG antibody against transglutaminase may indicate not only immune reactivity to the self-tissue but may assist practitioners in finding the root cause of these reactivities.

In fact, scientists have shown that antibodies against amyloid-beta-42 antibody reacted moderately to tTG3 and tTG6, but strongly to mTG. This is highly significant because mTG is found in numerous products that are consumed worldwide on a daily basis. If individuals react immunologically to mTG, the IgG antibodies produced could cross the barriers and bind to amyloid-beta peptide, contributing to amyloid plaque and neurofibrillary tangle formation and possibly the destruction of neurons.

4. PATHOGENS

Pathogens and their toxins not only can damage the gut and blood-brain barriers but also share a structural similarity with amyloid-beta and other brain tissues. Due to cross-reactivity, antibodies against these pathogens can damage these brain tissues and promote amyloid plaque formation.

- Oral Pathogens
- Enterococcus faecalis
- Escherichia coli and Salmonella CDT
- Campylobacter jejuni CDT
- Herpes Type 1

Elevated antibody levels against one or more of these pathogens and their toxins puts a person potentially at a higher risk for developing AD.

PATHOGENS

Interpretation of IgG antibody elevation against Pathogens

Detection of high levels of IgG antibodies against pathogens tested in this panel is usually indicative of chronic exposure to these microbes. These particular pathogens have been proven to play a role in the pathogenesis of AD: 1) they are found in the brains of patients with AD; 2) they share antigenic similarity or mimicry with amyloid-beta peptides; 3) pathogens and their antigens can bind to amyloid beta and cause its aggregation; and 4) antibodies produced against amyloid-beta peptides react strongly against them. Antibodies against oral pathogens, enteric bacteria (especially those that are producing endotoxins and cytolethal distending toxin), and Herpes Type 1 are very often detected in the blood of the normal population.

However, if these IgG antibodies are detected at high levels and cross the BBB, they may contribute to the pathogenesis of AD due to their reactivity with amyloid-beta peptide.

Interestingly, most of these pathogens and their toxins (oral pathogens, *Enterococcus faecalis*, *Escherichia coli*, and *Salmonella* CDT, *Campylobacter jejuni* CDT, Herpes Type 1) can damage the gut barrier and BBB, allowing the entry of antibodies, toxins, and the whole pathogen into the brain. Thus, detected antibodies against these pathogens and their role in amyloid plaque formation should be interpreted in the context of BBB breakdown. For example, high levels of IgG antibodies against Herpes Type 1 are observed in more than 50% of the population, due to the incidence of cold sores. However, during aging, trauma, and stress, the pathogens themselves cause the BBB to become compromised, and thus penetration of pathogen-associated IgG may contribute to amyloid plaque formation and neuronal degeneration.

5. CHEMICALS

Toxic chemicals or their metabolites can directly get into the brain and cause damage to the neurons. They can also combine with human proteins to form neoantigens and cause protein misfolding that mimics amyloid-beta aggregation, so that antibodies made against one will also attack the other, leading to neurodegeneration.

- Aluminums
- Dinitrophenyl
- Ethyl + Methyl Mercury
- Phthalates

Elevated antibody levels against one or more of these toxic chemicals or their metabolites indicates a person is potentially at a higher risk for developing AD.

CHEMICALS

Interpretation of IgG antibody elevation against Toxic Chemical bound to human tissue proteins

We included **Aluminums, Dinitrophenyl, Mercury** and **Phthalates** because research has found that after these chemicals bind to human tissue, they change the structure of the human tissue proteins into what looks like misfolded amyloid beta. The immune response against these new antigens (chemicals bound to human tissue, which are known as haptens) or the misfolded amyloid beta look-alikes results in IgG production against these new molecules. Unfortunately, it is not commonly known that after chemicals bind to human tissue, the human immune system reacts strongly to these haptens and **produces antibodies against the combination**. In fact, we develop allergies to penicillin based on this mechanism. Antibody production against penicillin occurs only after the use of the antibiotic and its binding to human carrier proteins in blood or in tissue. It is important to note that we do not make antibodies against chemicals such as penicillin themselves, but after they bind to human tissue, the chemicals and human proteins form a new structure that our immune system has never seen before.

Therefore, the detection of IgG antibody against these chemical-protein complexes in the blood indicates not only exposure to the chemical, but also repeated exposure and the binding of chemicals to human tissue proteins.

Because of the similarity between these misfolded proteins and amyloid beta, antibodies produced against chemicals bound to human tissue may also react with amyloid-beta peptide or its aggregates. That is why, for example, antibodies produced against mercury bound to human tissues will attack misfolded amyloid beta.

This antibody attack can enhance the process of autoimmune reactivity as well as neuronal degeneration in AD and other neurodegenerative disorders. In relation to AD, the detection of IgG antibody against any of these chemical complexes should be interpreted in the context of a compromised BBB, because chemicals or their antibodies, after crossing the barriers, bind to brain proteins and peptides that contribute to the degeneration of neurons.

6. FOODS THAT CROSS-REACT WITH AMYLOID-BETA

Many food proteins share a similar structure with the key brain protein amyloid beta, and antibodies produced against one can cause the immune system to attack both.

- Egg Yolk, raw + cooked
- Lentil & Pea Lectin
- Tuna, canned
- Hazelnut Vicilin + Cashew Vicilin
- Scallops + Squid
- Caseins
- Alpha-Gliadin + Gliadin Toxic Peptide
- Non-Gluten Wheat Proteins

Elevated antibody levels against one or more of these foods puts a person potentially at a higher risk for developing AD.

FOODS THAT CROSS-REACT WITH AMYLOID-BETA

Interpretation of IgG antibody elevation against Foods that cross-react with Amyloid-Beta

The detection of IgG antibody against food antigens is either wrongly interpreted as food allergy or completely dismissed. Since 2011, at Cyrex we have comprehensively investigated the role of food in autoimmunities resulting from molecular mimicry, cross-reactivity and other factors.

In relation to AD, the elevation of IgG in the blood should be interpreted first based on: 1) breakdown of the BBB; 2) an antibody's ability to cross the BBB; and 3) whether or not these antibodies react with amyloid-beta peptides through molecular mimicry.

We included these specific food antigens in Cyrex's Alzheimer's LINX™ because they were identified as the only foods out of 208 tested in a unique study to react with a specific antibody made against amyloid beta. This indicates that, due to a breakdown in the oral tolerance mechanism, a lack of digestive enzyme, or a possible leaky gut, food peptides or antigens can cross through the barriers, resulting in an immune response and the appearance in the blood of IgG antibodies against these food antigens. As long as the antibodies to these foods that cross-react with amyloid beta simply circulate in the blood, they may remain harmless. However, if they cross the BBB, they may come in contact with the amyloid-beta peptide and its aggregated forms, where they might further contribute to amyloid fibril and amyloid plaque formation.

Thus the detection of high levels of IgG antibody against these specific food antigens should not be interpreted simply in the context of food allergy or food intolerance, but as amyloid beta cross-reactive food antibodies and their possible role in neuroautoimmunity.

7. BLOOD-BRAIN BARRIER AND NEUROFILAMENTS

Production of antibodies against BBB proteins opens the neurological door to autoimmunity. A breach in the BBB increases the risk of allowing the entry of multiple autoreactive antibodies into the brain, contributing to brain tissue damage.

- Blood-Brain Barrier Protein + Claudin-5
- Aquaporins
- Neurofilament Proteins

Elevated antibody levels against one or more of these tissues puts a person potentially at a higher risk for developing AD.

BLOOD-BRAIN BARRIER PROTEINS

Interpretation of IgG antibody elevation against BBB Protein + Claudin-5, Aquaporins, and Neurofilament Proteins

BBB Protein, **Claudin-5**, **Aquaporin-4** and **Neurofilament Proteins** are major proteins involved in the structure and maintenance of the BBB. Production of antibodies against BBB protein opens the door to neurological disorders. When the BBB is intact, the presence of even multiple autoreactive antibodies in blood may not cause immediate harm, but a breakdown of the BBB increases the risk of antibodies reaching the central nervous system (CNS), contributing to neurodegeneration. This is why, when discussing IgG antibody elevation against proteins involved in AD, growth factors, enteric nervous system components, pathogens, toxic chemicals, and foods, we interpreted their importance in the context of a broken blood-brain barrier. Researchers have identified the BBB as a very crucial protective shield against neuroautoimmune disorders and related cognitive decline.

The detection of high levels of IgG antibodies against BBB Protein + Claudin-5, Aquaporins and Neurofilament Proteins may indicate a breakdown in the structure of the BBB, which may allow the entry of cross-reactive antibodies into the brain. Neurofilament antibodies may also reflect neuronal damage due to microglial (brain immune cell) activation. Elevated levels of these antibodies may increase the risk of developing AD and other neurodegenerative disorders.

CLAUDIN-5, AQUAPORIN AND NEUROFILAMENT PROTEINS

Interpretation of IgG antibody elevation against BBB Protein + Claudin-5, Aquaporins, and Neurofilament Proteins

Claudin-5 is a major cell-binding molecule and tight junction protein of brain endothelial cells. IgG production against it indicates damage to claudin-5 and other junction molecules such as occludin and zonulin. **Aquaporin-4** (AQP4) is a water channel protein that regulates water homeostasis in the CNS. Because AQP4 is located in astrocytic foot processes, antibody production against AQP4 may affect the feet of the astrocytes that protect the barrier.

Neurofilaments are intermediate filaments found in the cytoplasm of neurons. They provide structural support for axons or neurons and influence nerve conduction velocity.Antibody against neurofilaments is used as biomarkers of axonal damage in neurodegenerative disorders. The most common reason for neuronal damage is prolonged activation of brain immune cells, termed as **microglia**. When neurons degenerate, Neurofilament Proteins (NFP) are released into the blood where immune response against them result in antibody production. That is why, these antibodies could be elevated in several neurological disorders. Additionally, neurofilament proteins regulate the expression of other proteins that promote the regeneration of neurons, suggesting that antibody production against them could increase the risk for neuronal damage. Astroglia on the other hands are the most abundant fraction of glial cells. They protect the BBB integrity with processes or protrusions called end feet. Two major proteins of these end feet are AQP4 and **glial fibrillary acidic protein** (GFAP). When astroglia are compromised, overexpression of these proteins can trigger immune response and Antibody production to these proteins

Therefore, detection of high levels of IgG antibody against neurofilament proteins may indicate a breakdown in the BBB (astroglia overactivity), axonal damage (prolonged microglial activation), or both.

Overall production of high levels of IgG antibodies against BBB proteins may compromise BBB function during neurological diseases including stroke, traumatic brain injury (TBI) and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases and MS.

POSITIVE RESULT	NOTES
BRAIN PROTEINS	
Tau Protein	Increased risk for cognitive decline, or result of traumatic brain injury (TBI)
Amyloid-Beta Peptide	Increased risk for cognitive decline
Rabaptin-5 + Presenilin	Possible disruption of endocytosis and increased risk for cognitive decline
Alpha-Synuclein	Possible lack of neuroplasticity and increase risk for neurodegeneration
GROWTH FACTORS	
Beta Nerve Growth Factor	Possible lack of neuronal regeneration and increased risk for neurodegenerative disorders
Brain Derived Neurotrophic Factor	Possible lack of protection for neuronal cells and increased risk for multiple neurological disorders
Neurotrophins	Possible inability to protect and generate growth of neuronal cells and increased risk for neurological disorders
Somatotropin	Possible deficiency of growth hormone
ENTERIC NERVE, ENZYMES AND NEUROLOGICAL PEPTIDES	
Enteric Nerve + Vasoactive Intestinal Peptide	Increased risk for inflammatory enteric neuropathy and bowel motility dysfunctions
Transglutaminases	Increased risk for intestinal, skin and brain disorders
PATHOGENS	
Oral Pathogens	Increased risk for Alzheimer's disease, cardiovascular and arthritic autoimmunity
Enterococcus Faecalis	Increased risk for Alzheimer's disease and inflammatory bowel disease
Escherichia Coli CDT + Salmonella CDT	Increased risk for Alzheimer's disease, intestinal disorders and breakdown of the blood-brain barrier (BBB)
Campylobacter Jejuni CDT	Increased risk for Alzheimer's disease, intestinal disorders and breakdown of the blood-brain barrier (BBB)
Herpes Type-1	Increased risk for Alzheimer's disease, and cognitive decline
CHEMICALS	
Aluminums	Body burden of aluminum and its contribution to Alzheimer's disease
Dinitrophenyl	Body burden of dinitrophenyl and its contribution to Alzheimer's disease
Ethyl + Methyl Mercury	Body burden of mercury and its contribution to Alzheimer's disease
Phthalates	Body burden of phthalates and their contribution to Alzheimer's disease
FOODS CROSS-REACTIVE TO AMYLOID-BETA	
Egg Yolk (raw + cooked)	Immune reactivity to egg yolk and its cross-reaction with $A\beta_{42}$
Lentil Lectin + Pea Lectin	Immune reactivity to lentil and pea lectins and their cross-reaction with $A\beta_{42}$
Tuna, Canned	Immune reactivity to canned tuna and its cross-reaction with $A\beta_{42}$
Hazelnut Vicilin + Cashew Vicilin	Immune reactivity to hazelnut and cashew vicilin and their cross-reaction with $A\beta_{42}$
Scallops + Squid	Immune reactivity to scallops and squid and their cross-reaction with $A\beta_{42}$
Caseins	Immune reactivity to cow's milk caseins and their cross-reaction with $A\beta_{42}$
Alpha-Gliadin + Gliadin Toxic Peptide	Immune reactivity to wheat gliadin, its cross-reaction with $A\beta_{42}$, and the opening/destruction of intestinal tight junctions
Non-Gluten Wheat Proteins	Immune reactivity to non-gluten wheat proteins and their cross-reaction with $A\beta_{42}$
BLOOD-BRAIN BARRIER AND NEUROFILAMENTS	
Blood-Brain Barrier Protein + Claudin-5	Possible blood-brain barrier (BBB) breakdown of endothelial cells and tight junctions
Aquaporins	Possible blood-brain barrier (BBB) breakdown targeting astrocytes or food immune reactivity to corn, soy, spinach, tomato aquaporin
Neurofilament Proteins	Possible axonal injury and neurodegeneration

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