RATIONALE FOR COMBINATIONS OF NATURAL BOTANICALS AND COMPOUNDS FOR THE PROMOTION OF AGE-RELATED BRAIN HEALTH and PREVENTION OF COGNITIVE DECLINE.

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BACKGROUND.

The physiology of brain aging in women and men is associated with cellular and molecular changes that frequently result in impaired cognition, memory, mood and executive function.

The prevalence and degree to which this will occur depends on the timing and interplay of changes in the complex of multi- molecular factors associated with brain aging.

There are three potentially preventable and/or modifiable clinical outcomes. These need to be distinguished from the irreversible pathology of established dementia, with Alzheimer's Disease (AD) the main problem.

- Slowing of early recall with a decrease in concentration: synaptic dysfunction.
- Progressive decrease in short term memory and learning ability: benign senescent forgetfulness
- Abnormal molecular changes/metabolism : early Mild Cognitive Impairment (MCI)

The scientific formulation of our proposed *brain health promoting* products is based on established pharmacological actions of the individual ingredients, and prior "proof of concept " studies demonstrating their synergistic bioactivity when combined (US Patent : 6,524,616). Thus, the complementing mechanistic action of the ingredients on relevant physiologic molecular "checks and balance" results in a 1+1 =3 clinical outcome.

For example : by increasing the production of the memory "messenger" (the neurotransmitter acetylcholine) with soy estrogens (genistein) while reducing its natural inhibitor with huperizne A, more available synaptic acetylcholine results in an enhanced behavioral response , when compared to the effect of either ingredient on its own (US Patent 6,512,616).

PRODUCTS : CogniFemme ™ (for women) and CogniHomme ™ (for men)

Goals : Based on the above principle, two new patents have been filed (CogniFemme : PCT/ EP 2014/034549: CogniHomme 14/920,583) , with the goal of developing safe, effective and affordable products that address the "front end " of brain aging by raising the threshold of synaptic and neuronal function in otherwise healthy adults and to provide gender specific and age/condition adjusted formulations for those at risk of/or with symptoms of early cognitive decline and memory impairment.

THE SCIENCE: specifics are detailed in both patents and their respective claims (available electronically)

In short, by linking factors governing the molecular cascade of healthy and unhealthy brain aging with the established pharmacokinetics , pharmacodynamics and synergy of the combination's blend the following benefits are anticipated:

- **Neuronal Mass :** slowing of the "normal" age-related brain cell loss (apoptosis) and increase in the functional level of brain activity by stimulating neuronal precursor cells (stem cells) into mature neurons (neurogenesis) thereby reducing age related "neuropenia " and preserving cognitive reserve.
- **Neuronal Health**: improve brain cell health by modulating the metabolism of amyloid precursor protein and accumulation of harmful beta amyloid and hyperphosphorylated tau protein, plus reduce the added toxicity of inflammation and oxidative stress.
- **Neuronal Energy:** increase mitochondrial activity; maintain a healthy and protective blood brain barrier; increase brain blood flow and facilitate blood - brain glucose transfer, insulin sensitivity and brain glucose metabolism.
- **Neuronal Function:** enhance memory and cognition by up-regulating the key chemical messenger acetylcholine , modulating NMDA receptor activity and synaptogenesis.

THE INGREDIENTS: The cascade of physiologic and pathologic cellular changes and molecular pathways associated with normal and abnormal pathways, and the sites of the individual ingredients modulating activity is summarized in the accompanying attachment's figure. Sites of action of each ingredient indicated by a superscript number.

HUPERZINE A: Huperzine A is a well-studied alkaloid molecule whose structure was earlier defined by scientists at the Weizmann Institute of Science. (JAMA 1997; 277:776). Originally only available as an extract of the herb (Huperzia Serrata Trev) and used in Traditional Chinese Medicine (TCM) , it has since been successfully synthesized and scaled for production by scientists from Yale/Harvard and Sterling Pharma Solutions

The huperzine A molecule is highly brain specific and as such has a safe profile with few systemic side effects. It is a potent acetylcholine inhibitor with greater potency and fewer adverse effects than other currently available equivalents (e.g., Aricept ©.)

It is included in both CogniFemme ™ and CogniHomme ™ and although unable to retard or reverse neurodegeneration in patients with established AD, it does have multiple validated properties that stimulate neurogenesis; provide neuroprotection; facilitate neurotransmission and importantly, lessen the accumulation of both insoluble beta amyloid and hyperphosphorylated tau protein plus the frequently accompanying inflammation (huperzine non- cholinergic and combination Rx flow sheets).

The key to huperzine A's protective potential is its early use while viable and responsive neurons are available, plus its synergism with the bioactivity of the other ingredients in both product lines.

PHYTOESTROGENS: Genistein including its synthetic analog (for inclusion in CogniFemme ™)

Phytoestrogens are natural compounds that have estrogen like activity in humans. Included in this category are genistein , a principal bioactive component of our medical food for women..

Although genistein has a high affinity for the ER beta estrogen receptor similar to endogenous circulating natural 17 beta estradiol, it acts uniquely as an antagonist of the estrogen alpha receptor . Thus, it functions as a natural SERM (selective estrogen receptor modulator, equivalent to the drug raloxifene) by up-regulating estrogen like activity in the brain and bone and down-regulating the estrogen effect in the breast and endometrium (uterine lining).

Estrogen (genistein) has a number of accepted positive effects on brain neurophysiology and function: it regulates synaptic plasticity and potentiates synaptic transmission; increases the synthesis of BDNF (brain derived neurotrophic factor) a brain protein essential for synaptic formation and neurogenesis; modulates (with huperzine A) a key neurogenic molecular pathway (Wnt beta catenin); inhibits the beta secretase enzyme and the synthesis of insoluble beta amyloid (BACE inhibitor); has anti-inflammation and anti-oxidant properties; maintains the integrity of the blood brain barrier; increases cerebral blood flow; facilitates the transfer of glucose into the brain and promotes brain insulin sensitivity; stimulates acetylcholine synthesis; up-regulates the brain's vitamin D receptor and enhances its in situ synthesis of active vitamin D.

The acute decrease of estrogen following menopause corresponds to the earlier onset and greater prevalence of AD in women compared to men.

DHEA: Dehydroepiandrosterone (for inclusion in CogniHomme ™)

DHEA is a physiologic neurosteroid: neurons and their progenitor glial cells have endogenous enzymes for the local synthesis of DHEA and its various metabolites which via specific brain receptors have significant effects on neurogenesis, neuroprotection and cognitive function.

These actions include: regulation of neuronal death (apoptosis); stimulation of nerve growth factor (NGF) and BDNF synthesis; potentiation of synaptic transmission and plasticity; increase in axonal spine density with a resultant improvement in cognition; enhancement of acetylcholine synthesis; and most importantly, counteracting the negative effect on cognition of prolonged and excess levels of cortisol.

DHEA has proven benefits in experimental vascular dementia (VaD), consistent with cognitive improvement in patients with mild to moderate VaD treated with huperzine A. There are no reported studies with combination DHEA and huperzine A.

DHEA has vascular benefits in patients with impaired glucose metabolism and diabetes, significant risk factors for VaD and possibly for insulin resistant AD (type III diabetes).

Vitamin D: a systemic nuclear steroid (for inclusion in both CogniFemme ™ and CogniHomme™)

Although traditionally recognized as a "vitamin" synthesized in the skin and available from certain vitamin D rich foods, it is now well established that vitamin D is a member of the nuclear transcription regulator family with vitamin D receptors (VDR) present in most if not all tissues and organs.

In addition to abundant VDR's, the brain has enzyme systems that activate the synthesis of active vitamin D (AVD3) directly from precursors in the brain and is thus independent of vitamin D from the systemic circulation. This enzymatic conversion has been demonstrated in cells essential for cognition and memory: neurons, glial cells and activated microglial cells.

There are two main enzyme systems: one that increases VAD3 synthesis (CYP27B1) and another that enhances its breakdown (CYP24). The balance between the two determines AVD3's ultimate cellular activity.

Genistein up-regulates the VDR, its transcription and expression and in addition, by increasing the expression of CYP27B1 mRNA and suppressing CYP24 mRNA, facilitates vitamin D's in situ concentration and function.

Vitamin D has a number of neuroprotective actions : it regulates the synthesis of NGF (nerve growth factor);glial cell neurotrophic factor ; modulates calcium (Ca++) homeostasis (a critical factor involved in neurotransmission) ; increases the synthesis of acetylcholine ;inhibits activated microglia, the cells responsible for brain cell inflammation and death; enhances the brain to blood efflux of the harmful beta amyloid; and has a profound effect on insulin metabolism: it stimulates insulin receptor expression; enhances insulin responsiveness and glucose transport; increases the bioconversion of inactive pro-insulin to bioactive insulin.

Caffeine : an enhancing and synergizing functional additive .

Long term use of caffeine has been correlated with improved cognition and memory in later life and a lowered prevalence of dementia and AD. This can be attributed in part to its inhibition of acetylcholinesterase plus an increase in acetylcholine production. By freely crossing the blood brain barrier ,caffeine promotes an increase in hippocampal BDNF and dendritic branching and length.

Caffeine decreases the synthesis of beta amyloid via suppression of beta and gamma secretase expression and enhances brain amyloid clearance by upregulating the LRP1 receptor , which together with an increase in CSF production and cerebral blood flow, increases the efflux of beta amyloid from the brain.

Chronic use of caffeine decreases age related insulin resistance by enhanced conversion of pro-insulin to insulin; decreasing free fatty acids and by enhancing GLUT 4 , facilitates glucose uptake by muscle .

Going Forward From Lessons Learned: Applied Translational Medicine.

Post-menopausal osteoporosis was previously thought to be an inevitable consequence of aging. Women were destined to suffer the pain and loss of dignity of the so-called "dowagers hump", and the more serious potential of death, following hip fracture and pulmonary fat embolism.

With definition of the bone cell remodeling cycle and the ability to differentiate the treatable pre-condition, osteopenia (low bone mass; no fractures) from irreversible osteoporotic fractures, the latter is no longer an inevitable risk for aging women.

Our intent is to define "synaptopenia" and "neuropenia" in similar terms and by addressing this pro-actively prevent /modulate the progression of unhealthy brain aging and so the rapidly developing epidemic of age related cognitive impairment and dementia.

A number of interventions will be needed. Combinations of good nutrition, exercise and scientifically designed bioactive nutraceutical medical foods is our step in this direction.

This approach will be complemented by the use of validated surrogate brain blood biomarkers .

Based on the molecular changes associated with abnormal brain aging , these companion diagnostic tests will allow for both early identification of individuals at risk and as a means of monitoring response to treatment, similar to lipid profiling and the use of lipid lowering drugs.

Figure Legends :

1. **Huperzine A** : summary of the cholinergic and symptomatic treatment effects of huperzine A and its brain health protecting and disease modifying non-cholinergic actions. The latter forms the basis for the " new art " of the CogniFemme and CogniHomme products when used in combination with the other ingredients to complement and/or synergistically modulate brain health promoting signaling pathways and functions.

2. **Changes in Molecular & Cellular Pathways With Brain Aging.** The pharmacologic principle for both CogniFemme and CogniHomme is recognition of the multiple layers of factors involved in both "healthy" and "unhealthy " brain aging and to address them by incorporating the known " checks and balances " mechanistic bioactivity of the four ingredients. This includes neurotrophic factors (>synaptogenesis and > neurogenesis); neuronal health (<beta amyloid; <tau protein;<inflammation ; > beta amyloid efflux from the brain) while modulating the energy needs for brain function (>mitochondrial activity; > insulin sensitivity; > blood glucose

transfer and >BBB integrity); enhancing neurotransmitter synthesis and neurotransmitter activity (> acetylcholine and <NMDA acivity) and thus clinical outcome (cognition; memory; executive function).

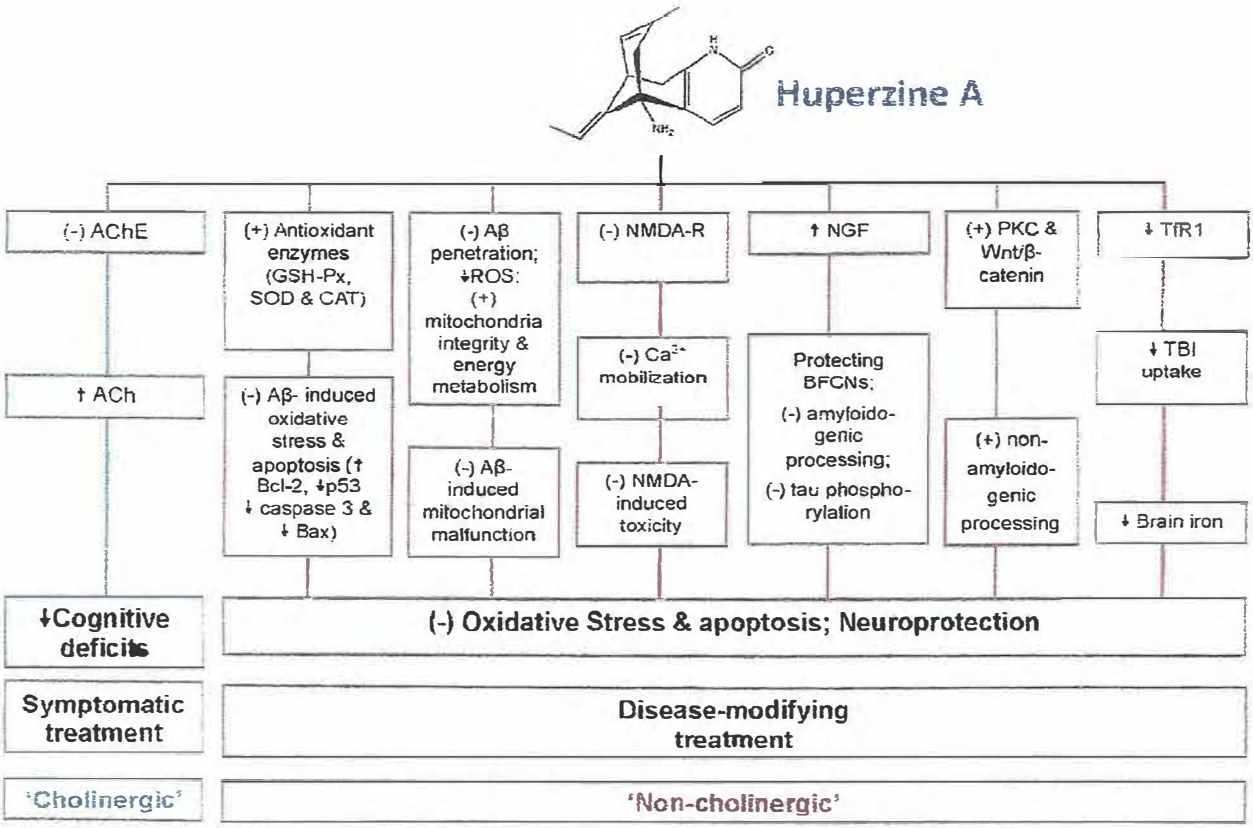
3. **Alzheimer's Disease Model and Points of Intervention (Cure Alzheimer Fund).** Addressing the three pillars for the pathogenesis of Alzheimer's Disease : Early stage (< amyloid accumulation); Mid-stage intervention (prevent tau tangles) and Late-stage (anti-inflammatory and <microglia). The subject invention is designed to modulate each of these steps (see text) mechanistically rather than the multiple associated risk factor genes.

4. **Subject Invention's Complimenting Non-Amyloidogenic Metabolism of Amyloid Precursor Protein :** Sites for the four ingredients complimenting and proposed synergism by increasing the neuroprotective sAPPa non-amyloid fraction (via >alpha secretase activity) and decreasing the insoluble beta amyloid sAPPb (via < beta secretase activity) ; and > beta amyloid clearance by both > its molecular breakdown and efflux.(see text). Assay of the blood platelet alpha secretase ;(sAPPa) beta secretase (sAPPb) and their ratios is the basis for the proposed companion diagnostic tests designed to identify persons at risk of MCI/Alzheimer's Disease and response to treatment with CogniFemme/Cognihomme .

5. **Adult Neural Stem Cell Neurogenesis: Subject Invention Ingredients and Stimulated Neural Pathways:** Stimulation of adult hippocampal neural stem cells into mature functional neurons has been validated and by balancing age related brain cell apoptosis , may maintain cognitive reserve and function. The sites for the subject inventions stimulation of the relevant signaling pathways are multiple and include two main pathways : > BDNF and CREB phosphorylation (synaptogenesis) and Wnt-Beta catenin (neurogenesis).

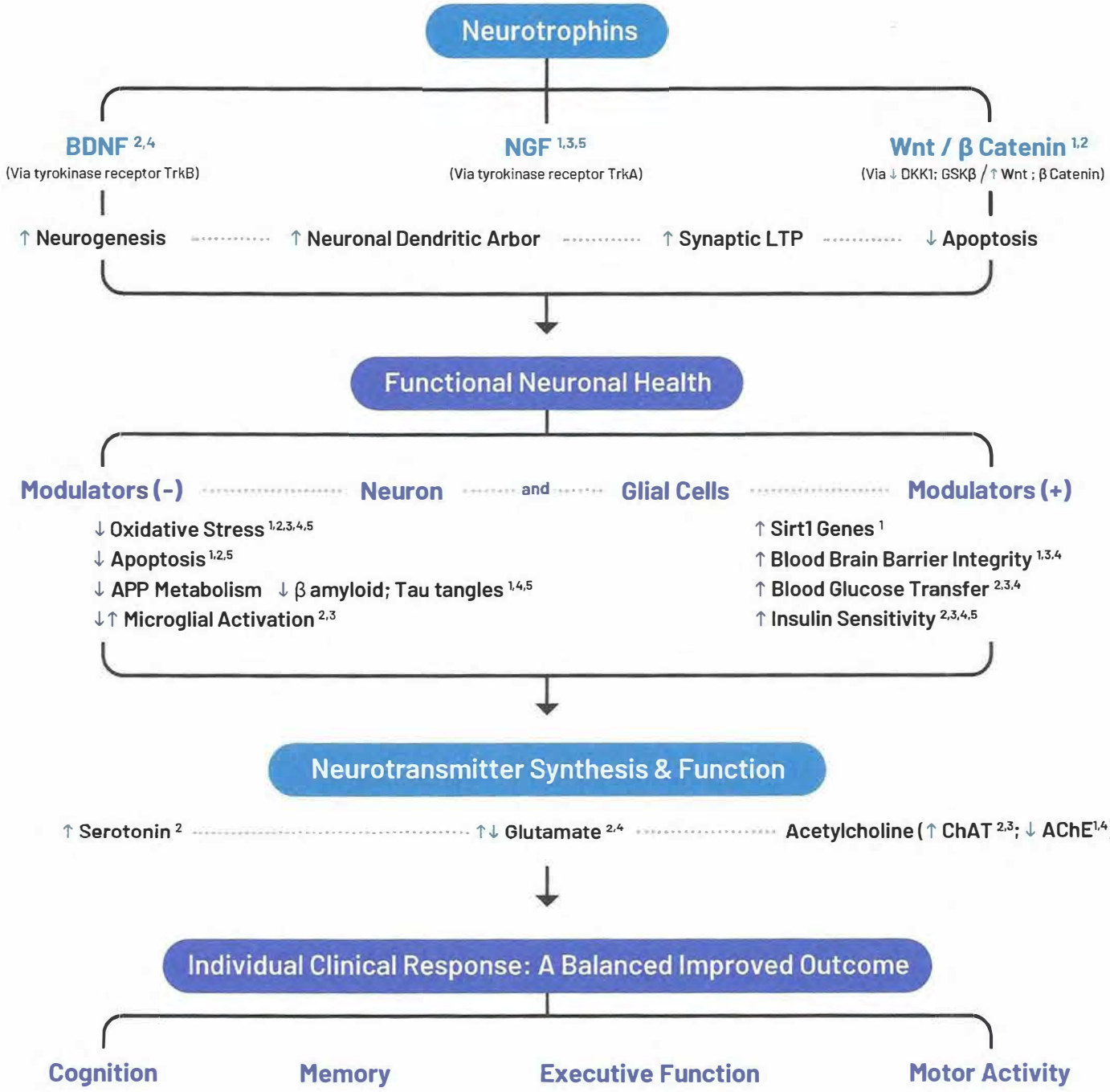
6. **Balancing the Regulation of Wnt/beta- catenin and DKK1 Signaling in the Brain :** This canonical pathway is a complex molecular pathway influencing significant brain health outcomes including synaptic connectivity; neurogenesis and neuronal health and survival. The validated and complimenting pharmacologic effects of huperzine and estrogen (genistein) are noted , with their potential synergism our claimed " new art" **APPLIED NOTES:** (a) huperzine A's inhibition of GSK 3beta : this is also a key factor in accumulated beta amyloid's phosphorylation of tau protein into neurofibrillary tangles. (b) Estrogen (genistein) inhibition of DKK1 : > blood levels of DKK1 has been associated with an increase prevalence of Alzheimer's Disease and vise versa. Assay of DKK1 (and BDNF) are included in our proposed brain health monitoring biomarker tests.

References : available electronically.


Huperzine A
(-) AChE
(+) Antioxidant enzymes (GSH-Px, SOD & CAT)
(-) Aβ penetration; ↓ROS: (+) mitochondria integrity & energy metabolism
(-) NMDA-R
↑ NGF
(+) PKC & Wnt/β-catenin
↓ TfR1
↑ ACh
(-) Aβ- induced oxidative stress & apoptosis (↑ Bcl-2, ↓p53 ↓ caspase 3 & ↓ Bax)
(-) Aβ-induced mitochondrial malfunction
(-) Ca2+ mobilization
(-) NMDA-induced toxicity
Protecting BFCNs; (-) amyloido-genic processing; (-) tau phospho-rylation
(+) non-amyloido-genic processing
↓ TBI uptake
↓ Brain iron
↓Cognitive deficits
(-) Oxidative Stress & apoptosis; Neuroprotection
Symptomatic treatment
Disease-modifying treatment
'Cholinergic'
'Non-cholinergic'

CONFIDENTIAL

Changes in Molecular & Cellular Pathways with Brain Aging and Sites of CogniFEMME™'s, Modulating Activity©



Sites of Activity

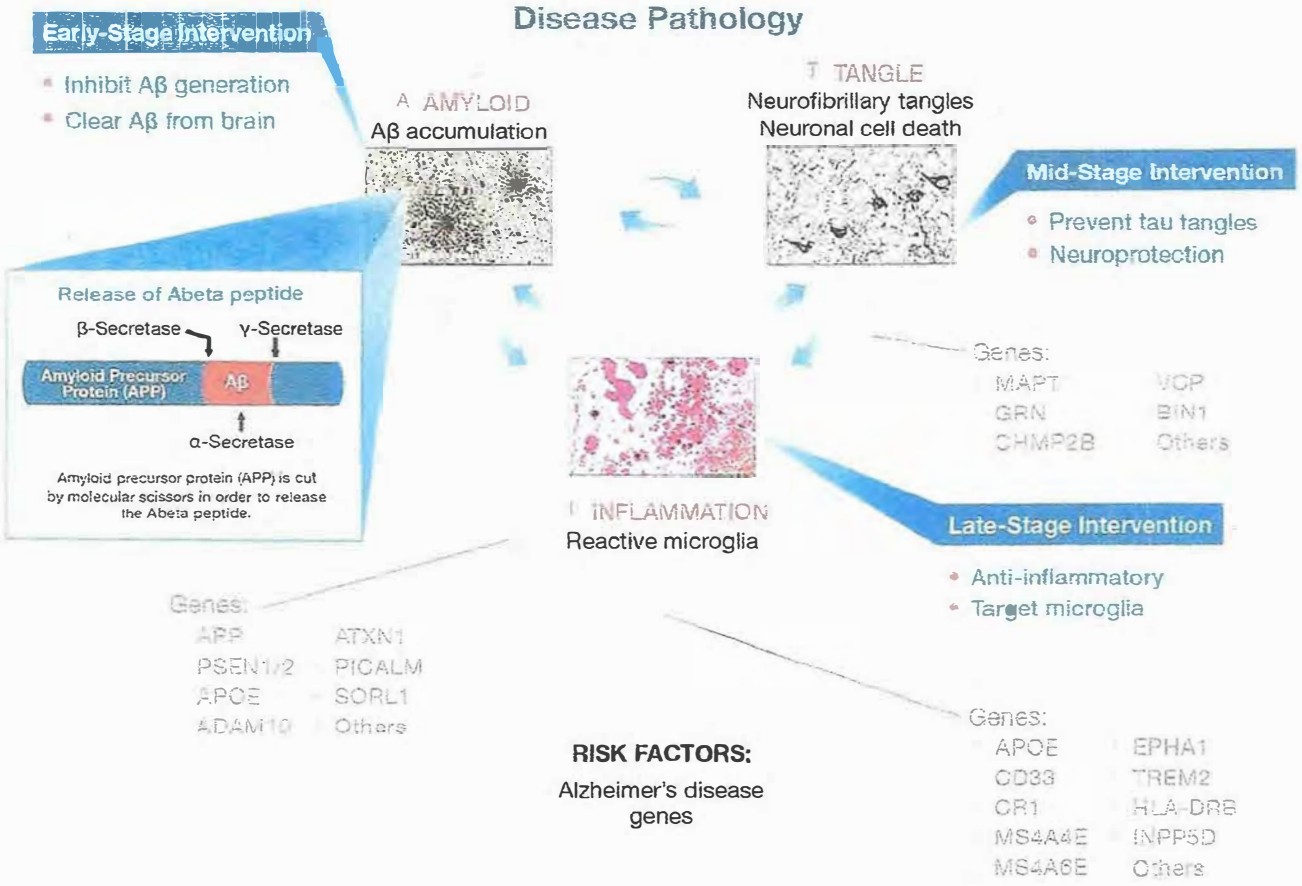
Huperzine A [1]
Soy Isoflavones [2]
Vitamin D [3]
Caffeine [4]
Sweetener/glp-1 [5]

Mechanistic Modulation

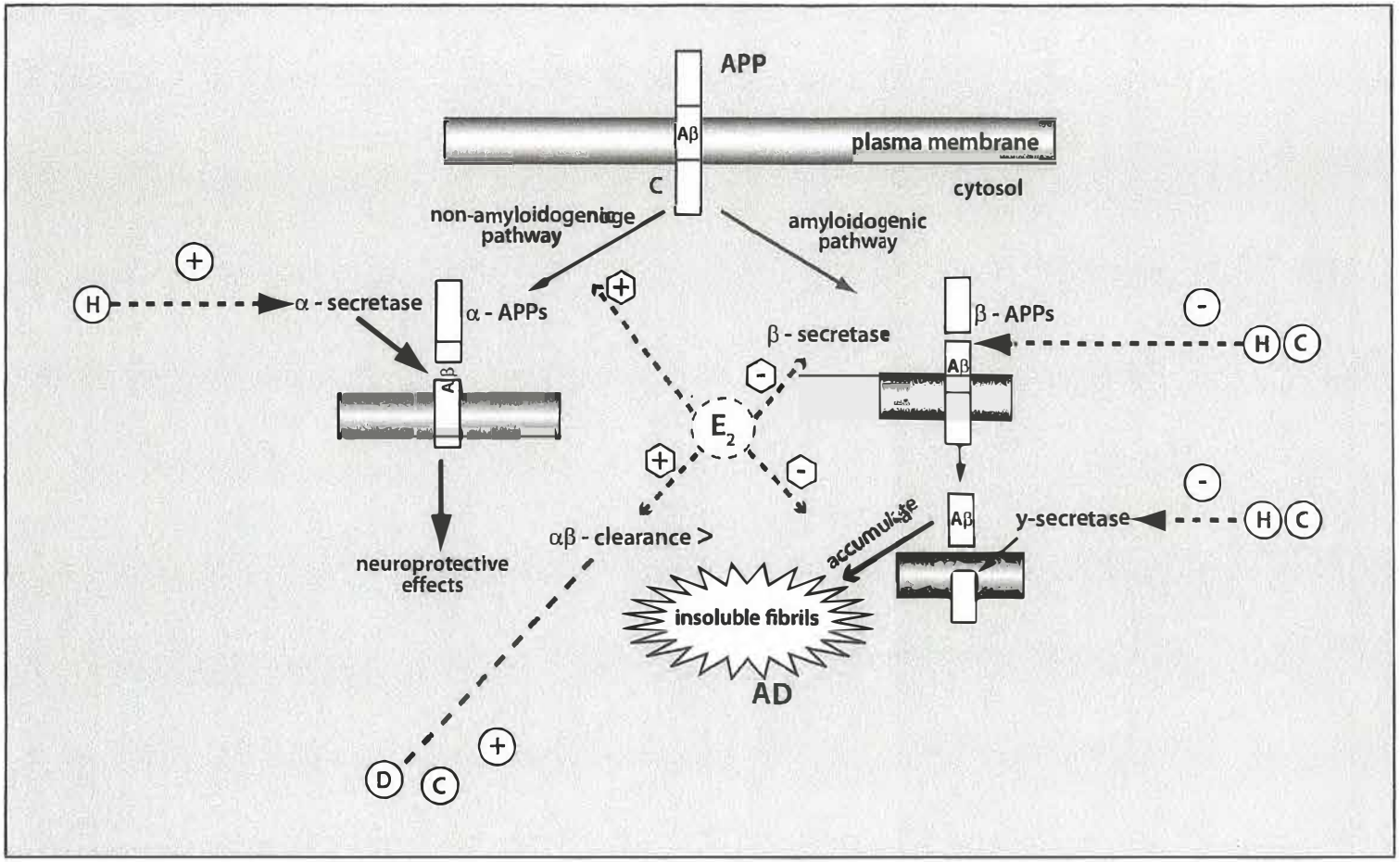
↓ Down Regulation
↑ Up Regulation
↓↑ Modulating Ratios

Alzheimer's Disease Model and Points of Intervention

RISK FACTORS:
- Traumatic Injury
- Menopause?
- Brain Infections?
- Neurotoxins?



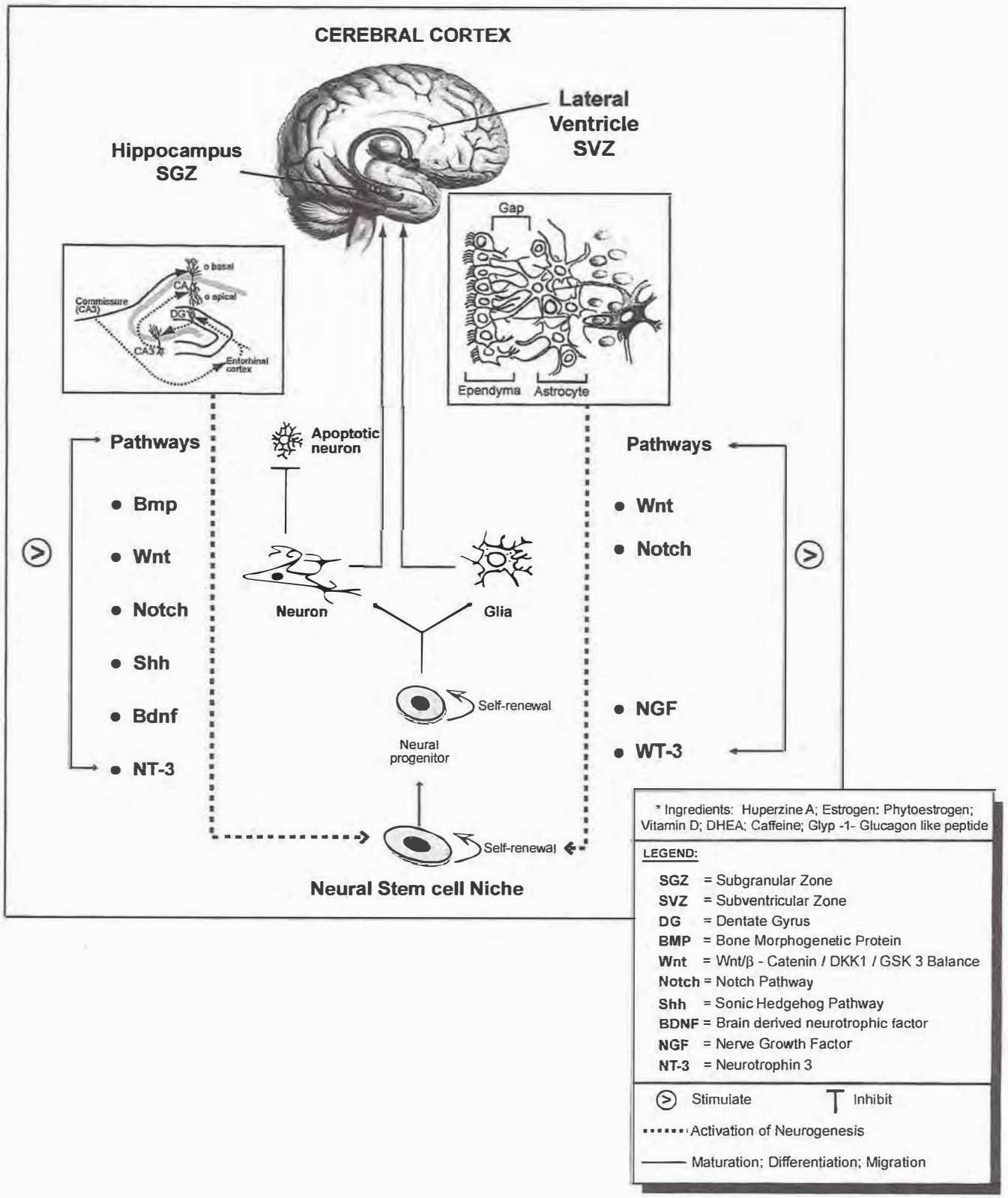
SUBJECT INVENTION'S COMPLIMENTING NON-AMYLOIDOGENIC METABOLISM OF AMYLOID PRECURSOR PROTEIN (APP)



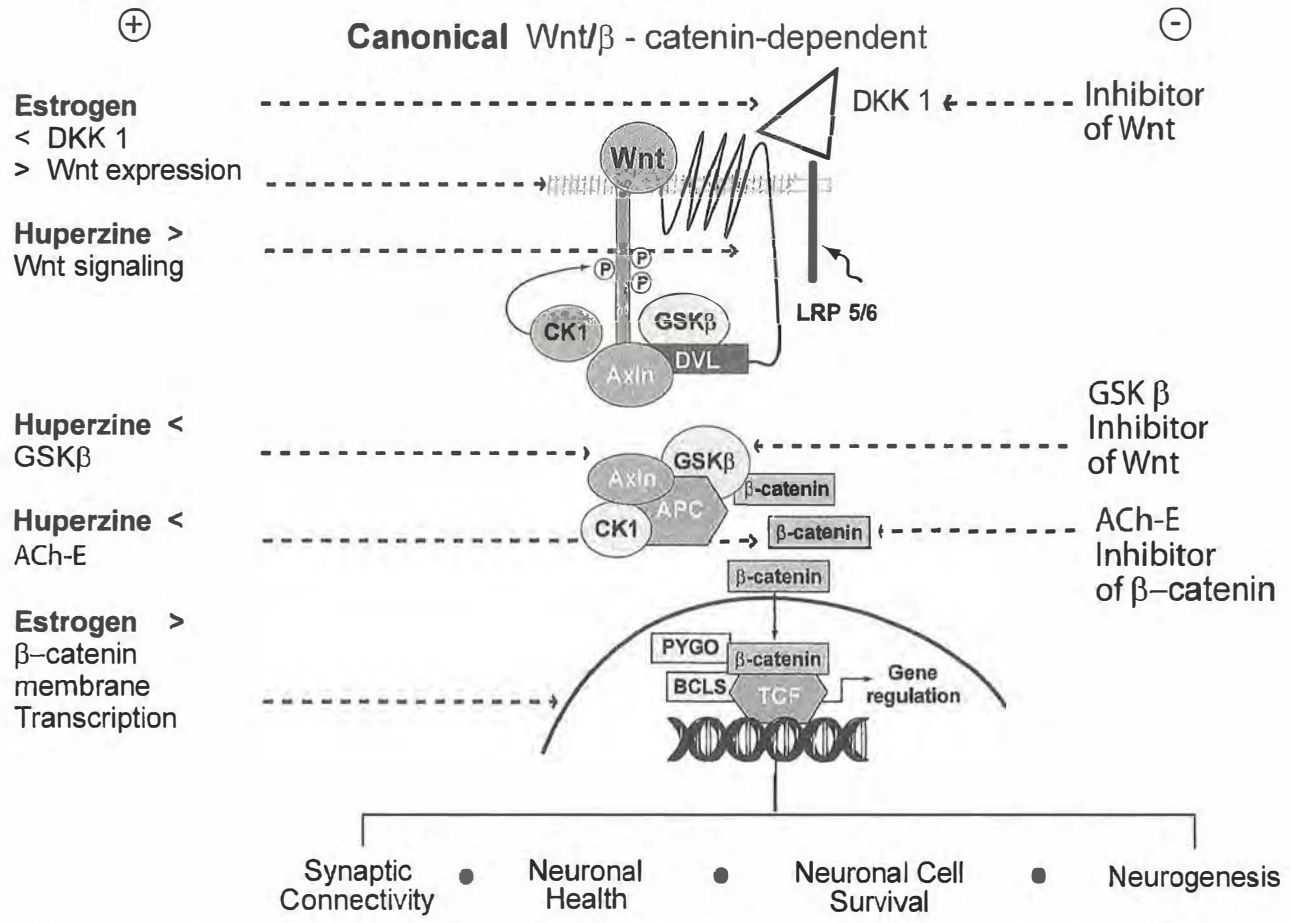
LEGEND: E_2 = estradiol; H = Huperzine A; D = vitamin D; C = caffeine; ⊕ = stimulates; ⊖ = inhibits
> = increases the clearance of solyble Aβ.

Cui et al (2013)

ADULT NEURAL STEM CELL NEUROGENESIS: SUBJECT INVENTION INGREDIENTS * AND STIMULATED NEURAL PATHWAYS



BALANCING THE REGULATION OF Wnt/β - CATENIN AND DKK1 SIGNALING IN THE BRAIN



Wang et al (2011) ; Scott and Brann (2013)