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DNA based customized nutraceutical “gene” therapy utilizing a genoscore: A hypothesized paradigm shift of a novel approach to the diagnosis, stratification, prognosis and treatment of inflammatory processes in the human

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Summary We hypothesize that using a multi-variant nutrigenomic index for the purposes of customizing or adjusting the formulation of nutritional supplements will result in an improved and novel approach to the diagnosis, stratification, prognosis, and treatment of inflammatory processes in the human. This multi-variant genetic index, or Genoscore, is derived by analyzing genotype and/or phenotype through measuring multiple genetic mutations of single nucleotide polymorphisms, gene expression, or other forms of genetic and phenotypic measurements. We also propose that manipulation of neurochemical reward circuitry in the mesolimbic brain region providing dopamine release at the nucleus accumbens (NAc), will have both pain and stress relief benefit, which are a cornerstone to the human inflammatory process. This hypothesis, applies to all genes currently discovered or which will be discovered and any nutritional or dietary supplement ingredient currently available or which will become available. For example, if a DNA test was measuring two genes through single nucleotide polymorphisms (Gene A and Gene B), the index scores (Genoscore) that would be reported to the clinician and patient would be based upon the number of mutations. An index score of 0 would mean no mutation. An index score of 1 may mean a mutation in Gene A. An Index Score of 2 may mean a mutation in Gene B. An Index Score of 3 may mean a mutation in Gene A and Gene B, resulting in a simple report, easily understandable to both the clinician and patient that provides insights into disease diagnosis, stratification, prognosis, as well as the metabolism, efficacy and/or toxicity associated with specific vitamins, minerals, herbal supplements, homeopathic ingredients and other ingredients in the nutritional and/or dietary supplement regimen. Furthermore, we have provided support that evidence shows the importance of the dopaminergic connection as an anti-pain and anti-stress molecule, working at the mesocorticolimbic region of

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the brain, specifically at the NAc. Additionally, we have provided support that clinical evidence demonstrates the effectiveness and safety of natural substances for joint health, such as glucosamine sulfate, chondroitin sulfate, and *Ganoderma lucidum*.

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36

37 Introduction

38 While often referred to as if it were a single dis-
39 ease, arthritis is actually an umbrella term used
40 for a group of more than 100 medical conditions
41 that collectively affect nearly 70 million adults
42 and 300,000 children in America alone. While the
43 most common form of arthritis – osteoarthritis
44 (OA) – is most prevalent in people over 60, arthritis
45 in its various forms can start as early as infancy.
46 Some forms affect people in their young-adult
47 years as they are beginning careers and families
48 and still others start during the peak career and
49 child-rearing years [1].

50 The common thread among these 100-plus
51 conditions is that they all affect the musculo-
52 skeletal system and specifically the joints –
53 where two or more bones meet. Arthritis-related
54 joint problems include pain, stiffness, inflamma-
55 tion and damage to joint cartilage (the tough,
56 smooth tissue that covers the ends of the bones,
57 enabling them to glide against one another) and
58 surrounding structures. Such damage can lead to
59 joint weakness, instability and visible deformities
60 that, depending on the location of joint involve-
61 ment, can interfere with the most basic daily
62 tasks.

63 For many people with arthritis, joint involve-
64 ment is not the extent of the problem. Many forms
65 of arthritis are classified as systemic. In these dis-
66 eases, arthritis can cause damage to virtually any
67 bodily organ or system, including the heart, lungs,
68 kidneys, blood vessels and skin.

69 Negative effects of arthritis

70 The disease also can affect other parts of the body.
71 Arthritis causes pain, loss of movement and some-
72 times swelling. Some types of arthritis that impact
73 joint health are:

- Osteoarthritis, a degenerative joint disease in which the cartilage that covers the ends of bones in the joint deteriorates, causing pain and loss of movement as bone begins to rub against bone. It is the most prevalent form of arthritis.

- Rheumatoid arthritis (RA), currently classified as an autoimmune disease in which the joint lining becomes inflamed as part of the body's immune system activity. Rheumatoid arthritis is one of the most serious and disabling types, affecting mostly women. New insights indicate that in RA, impaired galactosylation alters the requisite three dimensional configurations of glycoprotein structures, including certain immune factors, such as IgG and possibly type II collagen, producing the loss of self recognition and identity. This loss of self-identification alters recognition and response signaling during immune surveillance, inciting attack on the body's own joint collagen. Alterations of glycosylation and galactosyl structures are hallmark characteristics of RA. (Other autoimmune disorders have also been associated with faulty glycosylation.)
- Gout, which affects mostly men, is usually the result of a defect in body chemistry. This painful condition most often attacks small joints, especially the big toe. Fortunately, gout almost always can be completely controlled with medication and changes in diet.
- Ankylosing spondylitis (AS), a type of arthritis that affects the spine. As a result of inflammation, the bones of the spine grow together. Ankylosing spondylitis is also associated with a misrecognition event in which HLA-B57 is mistakenly identified as an unwelcome foreign antigen, which incites unremitting attacks that result in the characteristic damage of AS.
- Juvenile arthritis, a general term for all types of arthritis that occur in children. Children may develop juvenile rheumatoid arthritis or childhood forms of lupus, ankylosing spondylitis or other types of arthritis.
- Systemic lupus erythematosus (lupus) is a serious disorder that can inflame and damage joints and other connective tissues throughout the body.
- Scleroderma is a disease of the body's connective tissue that causes a thickening and hardening of the skin.
- Fibromyalgia, is where widespread pain affects the muscles and attachments to the bone. It affects mostly women.

Demographics

According to the United States Arthritis Foundation, arthritis and related conditions are a major cause of disability in the United States, costing the US economy more than \$124 billion per year in medical care and indirect expenses such as lost wages and production – and costing millions of individuals their health, their physical abilities and, in many cases, their independence. And unless something changes, the picture is going to get worse. As the population ages, the number of people with arthritis is growing.

Arthritis is one of the most relevant chronic health problems, and in America it is the leading cause of disability among Americans over age 15. Arthritis is second only to heart disease as a cause of work disability. Arthritis limits everyday activities such as walking, dressing and bathing for more than 7 million Americans. Arthritis results in 39 million physician visits and more than a half million hospitalizations in the United States alone. Costs to the US economy totals more than \$86.2 billion annually. Baby boomers are now at prime risk. More than half those affected are under age 65. Half of those Americans with arthritis don't think anything can be done to help them. Arthritis strikes women (41 million) more often than men (almost 29 million).

Standard of care treatments: pharmacoeconomics

There are over 20 million osteoarthritis sufferers in the United States alone [1]. Ten million of those sufferers are aging baby-boomers between 40 and 60 years of age who are still active, employed, and caring for their families [2]. This patient segment has been referred to as the "Boomeritis" segment [3]. Many of these patients self-medicate themselves. Many "Boomeritis" patients take over the counter anti-inflammatories and painkillers such as ibuprofen and acetaminophen, which is a segment in excess of \$10 Billion [4]. Interestingly, a higher proportion of these baby-boomers (44% of them) also take alternative therapies to address their healthcare concerns, indicating that traditional pharmaceuticals may not fully address their discomfort [5]. Many of these patients were taking COX-2 inhibitors for their inflammation because the marketing messages and data suggested that they were safer. With \$5 Billion worth of Merck's rofecoxib and Pfizer's valdecoxib pulled from the market, and new warnings added to the remaining

anti-inflammatories on the market about side effects, such as cardiovascular complications, a huge void has been left with these patients. Most arthritis patients seek a combination of centrally-acting agents to treat their pain, such as acetaminophen, codeine, or hydrocodone; along with anti-inflammatory agents such as ibuprofen, naproxen, or celecoxib.

Mechanism of action – centrally-acting neurotransmissions

Dopamine and pain: brain reward cascade

The principle ascending pathways for pain (e.g. spinothalamic tract) originate mainly in the dorsal horn of the spinal cord and medulla wherein second order neurons receive synaptic input from primary afferent neurons that supply nociceptors in tissue. The second order neurons of origin are within layer I as well as deep layers (IV–VI) of the dorsal horn [6]. Second order neurons of origin of pain-related pathways are mainly wide dynamic range (WDR) neurons or nociceptive-specific (NS) neurons and these two types of neurons process both exteroceptive and interoceptive information associated with pain. Our cutaneous nociceptive system clearly serves an exteroceptive role in signaling potentially dangerous stimuli impinging upon our bodies, so that we can respond appropriately, depending upon the situational context. Our interoceptive nociceptive system signals tissue disorders (e.g. rheumatoid) that are essentially inescapable, and calls for responses more obviously in the homeostatic domain.

Pharmacological aspects of pain control

Opioids such as morphine and heroin and psychostimulant drugs such as amphetamine and cocaine are effective pharmacological tools against chronic pain. Interestingly, amphetamine and related drugs relieve cancer pain and sometimes administered as an adjuvant analgesic in the clinical situation because they potentiate opioid analgesia and counter opioid-related sedation and cognitive disturbances. In support of these clinical findings, studies have shown that, in rats, psychostimulants potentiate the analgesic effect of morphine in an animal model of persistent pain [7]. There is increasing evidence that sites rostral to the brainstem play a critical role in the analgesic effects of opioid and psychostimulant drugs. It is well known that opioids can inhibit pain by acting at spinal sites and at sites in the brainstem where they modulate activity in descending brain stem pathways projecting to the

spinal cord. A primary site of action is the periaqueductal gray of the brain stem, where stimulation of opioid receptors activates through direct projections, serotonin-containing cells in the nucleus raphe magnus. In turn, the latter cells activate neurons that project, via the dorsolateral funiculus, to the dorsal horns of the spinal cord where they inhibit cells that transmit information about noxious painful stimulation from the periphery to supraspinal sites. The brainstem – descending pain-suppression system, however, plays a more important role in the suppression of brief, rapidly rising, transient, and well-localized (i.e. phasic) pain than it does in the suppression of injury-produced persistent (i.e. tonic) and inescapable pain. However, several lines of evidence suggest that the inhibition of the tonic pain requires the activation of neural systems in addition to those required for inhibition of phasic pain [8].

Mesolimbic dopamine in the suppression of tonic pain

There is little information to date concerning the identity of the endogenous pain systems that serve to inhibit tonic pain. The suppression of tonic pain involves systems in addition to those known to suppress phasic pain, and that these systems appear to involve forebrain sites, rostral to the brainstem. A clue to this problem is that both opioids and psychostimulants reduce tonic pain and increase transmission in mesocorticolimbic dopamine neurons known to be activated by natural rewards such as food and sex. These neurons arise from dopamine cell bodies that lie in the ventral tegmental area (VTA) and project to various forebrain sites such as the nucleus accumbens (NAc), amygdala, and prefrontal cortex. Opioids cause the release of dopamine from these neurons through their indirect activation (see reward cascade), whereas psychostimulant drugs such as amphetamine and cocaine increase dopamine extracellularly by decreasing reuptake and/or inducing release. Moreover, opioids and psychostimulants have both rewarding effects and analgesic effects in the clinical setting, suggesting that reward and analgesia might share common neural substrates [9]. Morgan and Franklin found that dopamine-depleting 6-hydroxydopamine lesions of the ventral midbrain, which contains the cell bodies of the neurons that give rise to ascending forebrain projections, block the analgesic effects of systemic morphine and amphetamine in the formalin, but not the tail-flick test [10,11]. Their findings provided the first evi-

dence that mesolimbic dopamine neurons play a role in the suppression of tonic, but not phasic pain. In recent studies, Taylor et al. found that while the D1-selective agonist SKF 38393 was without effect at a dose of 0.5 nmol/side, the D2-selective agonist quinpirole dose dependency (0.05–5.0 nmol/side, bilateral) inhibited the persistent phase of formalin-induced nociception [12]. This was blocked by pre-administration of a selective D2-dopaminergic antagonist raclopride. These results indicate dopamine agonists that activate D2 receptors in the NAc, inhibit inflammatory pain.

Dopamine D2 receptors and chronic pain

Interestingly the dopaminergic system and specific manipulation of this pathway that changes synaptic neurotransmission in the brain are thought to play a role in chronic pain. Animal studies suggest that striatal and cortical dopaminergic systems participate in pain transmission or modulation. Dopamine D2 receptors have been reported to mediate the inhibitory role of dopamine in animal models for persistent pain [13]. Hagelberg et al., showed that in healthy volunteers that high D2 receptor availability in the putamen is associated with low cold pain threshold and a high pain modulation capacity induced by conditioning stimulation [14]. Furthermore, decreased [18F] FDOPA uptake and increased D2 receptor availability have been demonstrated in the putamen in a chronic orofacial pain state, the burning mouth syndrome. Moreover, it was found that the increase in D2 receptor availability in the left putamen and the decrease in D1/D2 ratio imply that alterations in the striatal dopaminergic system as evaluated by PET may be involved in chronic orofacial pain conditions. In essence, we hypothesize that low or hypodopaminergic function in the brain may predispose individuals to low pain tolerance. Current research would support this concept and thus carriers of the D2 *Taq A1* allele as observed in reward deficiency syndrome (RDS) behaviors may be good candidates for nutrients designed to enhance dopamine release in the brain and may impact the inflammatory process [15].

Stress in America

The effects of excessive stress in modern life leads to chronic states of fatigue-related depression. This is an unfortunate fact yet true that about 80% of all illness can be traced back to stress and depression. The American Academy of Family Physicians suggests that about 2/3 of office visits relate to stress.

Stress and dopamine: implications for the pathophysiology of chronic widespread pain

The relationship between stress, endorphins and hypothalamic-pituitary-adrenal (HPA) axis is well known [15–17]. Certainly in the world of addiction stress plays a critical role in both the acquisition and relapse. It is known that certain genetic and environmental elements play significant roles in drug dependency and dysregulation of brain reward pathways. In fact, dopamine D2 receptor polymorphisms have been associated with stress coping mechanisms and posttraumatic stress disorder [18]. Interestingly, either stress can induce a painful condition or it can exacerbate the pain. Exposure to stress also activates dopamine transmission in mesocorticolimbic dopamine neurons [19] and this effect appears to involve opioid mechanisms in the VTA. More specifically, intra-VTA infusions of the opioid receptor antagonist Naltrexone, prevent the stress-induced activation of dopamine metabolism in the NAc and prefrontal cortex, and exposure to stress causes the release of met-enkephalin into the VTA [17]. These findings combined with those indicating that exposure to stress can inhibit tonic pain and that intra-VTA morphine induces analgesia in the formalin test, suggest that the endogenous release of opioids in the VTA might be a mechanism underlying the stress-induced inhibition of tonic pain. This has been supported by the finding that intra-VTA infusions of the opioid receptor antagonist, Naltrexone, stress-induced analgesia in the formalin test [11,20,21]. In addition, it has been proposed that release of the tachykinin neuropeptide, substance P (SP), in the VTA might play a similar role in the stress-induced suppression of tonic pain. In this regard, Altier and Stewart have also found that activation of midbrain dopamine neurons by SP did indeed inhibit tonic pain in the formalin test [9]. The current data suggests that exposure to stress induces analgesia by causing a release of SP in the VTA, which in turn activates mesocorticolimbic dopamine neurons. Finally, opioids, amphetamine, and SP all share the ability to increase dopamine release in the NAc. Moreover, opioids administered systemically or into the VTA augment dopamine metabolism and extracellular levels of dopamine in the NAc.

With that background it becomes increasingly clear that tonic pain may be attenuated by dopamine D2 activation. It follows then that we embrace a natural method to cause a preferential release of dopamine in mesocorticolimbic pathways. In this regard, support of an attenuation of stress has been found with a variant of the Synap-

tamine™ complex in a double-blind placebo controlled study [22]. The Synaptamine™ complex is a patented natural supplement that contains certain neurotransmitter precursor amino-acids, trace metals and herbals (Salugen, Inc. San Diego, California). We further hypothesize herein that unless there is a way of increasing endogenous opioids, which in turn inhibit GABA causing dopamine release in the NAc, simple neurotransmitter precursors will not be as effective in reducing tonic pain.

Fibromyalgia

One example of how stress, pain and dopamine may interact involves fibromyalgia (FM), which has been called a "stress-related disorder" due to the onset and exacerbation of symptoms in the context of stressful events [20]. The cardinal feature of FM is pain, the experience of which involves both afferent and efferent processes. While exposure to acute stress is known to produce stress-induced analgesia (the induction dependent upon dopamine containing neurons within the NAc), rat studies have demonstrated that prolonged exposure to stress eliminates this response, resulting instead in a state of stress-induced hyperalgesia [21]. Chronic stress has been shown to result in the attenuation of dopaminergic activity within the NAc and is therefore proposed to contribute to the development of stress-related hyperalgesia.

Interestingly, in FM patients clinical studies have suggested a disruption of dopaminergic function, including but not limited to decreased dopamine metabolites in cerebrospinal fluid [22,23]. A variety of stressors result in the release of dopamine within the NAc, including acute psychological stress a cornerstone symptom of FM [24]. Thus, a vicious cycle occurs whereby stress from the pain further exacerbates the release of dopamine, which in turn results in a hyperalgesia state. Hyperalgesia to both thermal and chemical stimulants persists up to 9 days after stress exposure in rats [25]. Moreover, other neurotransmitters are also involved as well. The selective 5-HT reuptake inhibitors clomipramine and fluoxetine, as well as the 5-HT reuptake precursor tryptophan, blocks development of hyperalgesia, suggesting that repeated stress produces a long-lasting increase in pain sensitivity. In fact, whereas there is a disruption of both serotonergic and dopaminergic function that occurs within the NAc following chronic stress, the impact on dopamine outlasts that of 5-HT. In this regard there are three possibilities which have been proposed: (1) there is regulatory interaction between 5-HT and dopamine during stress-induced analgesia; (2) a disruption of this

interaction contributes to the inception of stress-induced hyperalgesia; and (3) dopaminergic dysfunction, which outlasts that of 5-HT, may be responsible for the persistent expression of stress-induced hyperalgesia after serotonergic function has been normalized. This phenomenon may explain why strategies aimed at boosting serotonergic function only on patients with chronic widespread pain have met with limited success insofar as analgesia is concerned. Thus since FM is a stress-related disorder, one would predict that strategies aimed at boosting dopaminergic function within the mesolimbic pathway would have superior efficacy. While no one has attempted combining therapies in term of multiple pharmacogenomic targets, and the outcome of such an attempt is unknown, thus we are hypothesizing that natural manipulation of the reward signaling and circuitry could potentially become an important therapeutic modality [26–28]. Breaking of this cycle with a stress reducing natural enkephalinase and catecholamine-*o*-methyltransferase (COMT) inhibitors such as Synaptamine™ with a genetically-customized formulation of nutritional supplements, is clearly warranted.

Mechanism of action: local inflammation and joint health

In addition to the centrally-acting agents, joint health can be supported locally at the site of inflammation. The most common pharmaceuticals used for arthritis are non-steroidal anti-inflammatory products, such as ibuprofen and naproxen. Many nutritional supplements have demonstrated in clinical studies to reduce the signs and symptoms of joint damage, such as pain and inflammation, as well as commonly used over the counter pharmaceuticals, such as ibuprofen. One such nutritional supplement is glucosamine.

Glucosamine is an aminomonosaccharide, a component of almost all human tissues, including cartilage. It is the principle component of O- and N-linked glycosaminoglycans, which form the matrix of all connective tissues. Glucosamine sulfate has a relatively low molecular weight and is the sulfate salt of the natural aminomonosaccharide, glucosamine. Glucosamine is commercially available in pharmacies, health food stores, and retail stores and is sold via the Internet. It is most commonly available as the sulfate, HCl, N-acetyl or chlorhydrate salt isomers, which are water-soluble [29]. The sulfate and HCl forms differ in their purity, sodium content, bioactive glucosamine, and equivalent dosages. Unlike glucosamine sulfate and HCl

forms that are most commonly used in clinical trials, glucosamine does not have active intestinal transport.

Glucosamine sulfate provides pain relief and improved function in knee OA [30]. In a recent 3-year, randomized, placebo-controlled, prospective study by Bruyere et al., 212 patients with knee OA were evaluated to determine the effect of glucosamine and chondroitin on symptom and structure modification in knee OA [31]. In patients who had mild OA and were in the highest quartile of baseline mean joint space narrowing, glucosamine was associated with a trend ($p = 0.10$) towards a significant reduction in joint space narrowing [32]. The authors reported indistinguishable symptomatic efficacies for both compounds as indicated by two 3-year, double-blinded, controlled studies [33,34].

Another nutritional supplement used frequently by arthritis sufferers is chondroitin sulfate, which occurs naturally in human cartilage, bone, cornea, skin and the arterial wall. Preparations of chondroitin sulfate are derived from bovine and calf cartilage. Chondroitin sulfate is a larger and more poorly absorbed; <10% intestinal absorption compared to 90% for glucosamine sulfate [35]. We hypothesize that this poor absorption may be due to more than its formulation, but that genotypic and phenotypic factors may contribute to its poor absorption in certain patients.

Several small, short-term, 3- to 12-month, randomized placebo controlled clinical trials to evaluate the effects on chondroitin sulfate/placebo or NSAID have demonstrated modest reductions in knee OA pain and improved function [35]. Sustained effects have been reported up to 3 months after discontinuation of chondroitin sulfate [36].

Reports of small, randomized controlled trials have examined the combination of glucosamine and chondroitin sulfate for knee OA pain and low back pain [37]. Muller-Fastbender et al. found that glucosamine sulfate was as effective as ibuprofen in reducing joint damage with fewer side effects than ibuprofen [38]. Mazzieres et al. found similar results when comparing chondroitin sulfate and ibuprofen [39].

Some recent data presented at the 2005 Annual Meeting of the European League Against Rheumatism (EULAR) supports these earlier clinical findings. Abou-Raya et al. [40] found some interesting evidence relating glucosamine sulfate use and improved clinical results, and reduced anti-inflammatory usage. Their study consisted of 100 patients, mean age 63.5 years of age, with primary knee OA diagnosed according to the ACR criteria for diagnosis of primary OA of the knee. Knee OA was documented by radiographic examination using

the Kellgren–Lawrence grading scale – radiological stage I–III were selected. Symptomatic OA was defined as the need to take non steroidal anti-inflammatory drugs (NSAIDs) daily and Lequesne Algofunctional Index (LFI) score >4 and <14 . Patients who fulfilled these criteria were entered into a double-blind, randomized, placebo-controlled trial for a 6-month treatment period. Clinical assessment using the Visual Analogue pain Scale (VAS, 0–100 mm), the Lequesne Algofunctional Index (0–24) and the WOMAC functional index were conducted at baseline and at the end of the study. The patients were divided into two groups; 50 patients were allocated to receive 1500 mg, $3 \times$ daily of alpha-[D]-glucosamine sulphate (GS), an active isomer of GS; and 50 patients received a placebo. A 15 day washout of NSAIDs preceded the study. NSAIDs were allowed after entry into the study as required. At the end of the study, the analysis demonstrated that the mean LFI score decreased from 9.2(1.2) to 6.7(1.0) in the GS group and from 9.0(1.0) to 8.6(0.8) in the placebo group ($p < 0.001$). A significant change was noted in the VAS; pain decreased from 55.0(12.8) to 39.4(11.4) mm in the GS group and from 53.9(11.4) to 49.2(10.5) mm in the placebo group ($p = 0.001$). Functional disability was significantly reduced in the GS group. WOMAC scores improved significantly in the GS group compared to the placebo group ($p < 0.001$). Fewer patients in the GS group required analgesics (39% versus 68% in the placebo group). Tolerance was good to very good for most patients. Side effects included GI upsets in the form of dyspepsia (11%) and increased GI motility (6%). In conclusion, this data revealed that GS was superior to placebo in several aspects: improvement of the LFI, VAS and reduction of NSAID/analgesic consumption. This study demonstrated the efficacy of the symptomatic slow acting drug, GS, in reducing pain and improving function in patients with knee OA. Thus, it is feasible to include GS in the therapeutic armamentarium of OA to retard progression of the disease, relieve symptoms, improve function and consequently improve the quality of life of OA sufferers. We hypothesize that the efficacy of GS can likely be enhanced based upon a prospective patients nutrigenomic profile and thus better targeting patients and their respective doses will better predict who may be more responsive to therapy.

In a cohort of 184 patients suffering from knee OA, Kamel et al. [41] found demonstrable evidence that glucosamine sulfate (GS) and chondroitin sulfate (CS) impact joint health. The average age 52 years was studied. A control group of 100 OA patients of a matched age and clinical stage

was included. The patients received 1500 mg of glucosamine sulphate and 1200-mg chondroitin sulphate daily for 24 months after informed consent. The control group continued their classical treatment without taking GS. The HDI 3000 ATL US machine, equipped with 5–12 MHz linear transducer was used. MRI 0.5 T using axial T1/wt, T2/.wt axial Fat saturated FSE and coronal or sagittal fast STIR sequences were used to measure the thickness of the articular cartilage at baseline, 6 months, 1 year and 2 year following glucosamine treatment. Images were arranged in random order and quantified by reader blinded to time sequence. MRI images were analyzed for the presence and severity of cartilaginous lesions, surface irregularities, subchondral cyst and bone marrow edema. Femoral articular cartilage thickness of medial and lateral compartment was assessed by ultrasound and MRI with a validated computerized algorithm. Pretreatment cartilage defects; diffuse cartilaginous thinning, partial thickness or full thickness irregularities with and without subchondral cyst and/or sclerosis and bone marrow edema, were the most common findings seen in OA knee cartilage of the study population. Following GS treatment, both ultrasound and MRI images showed a significant improvement in the quality of the three layers of knee articular cartilage (superficial, intermediate and deep layers) in 78% of patients. The cartilage outlines became well defined in 65%. The echotexture showed a significant decrease of echogenic white dots in 81%. The diffuse cartilage thinning of both medial and lateral compartments had a substantial increase of thickness compared to pretreatment measurement in 76% of patients. The post-treatment recorded articular cartilage measurements were statically significant compared to the controlled group $p < 0.5$. This study provides reliable imaging evidences for the effectiveness of glucosamine sulphate treatment in patients suffering from knee osteoarthritis. However, similar to pharmaceutical studies, some patients did not respond – a portion (22%) did not show significant improvement in the three layers of knee articular cartilage, and a portion of patients did not experience an increase in thickness (24%). We hypothesize that nutrigenomic factors may contribute to the response to GS and CS and that by analyzing these factors, a patient can better choose between supplements and the respective doses.

Other nutritional supplements have also demonstrated potential efficacy in the treatment of signs and symptoms of arthritis, such as pain, inflammation, and joint damage. Those all-natural ingredients include quercetin [42], *Ganoderma lucidum*

[43], bromelain [44], *dl*-phenylalanine [45], and gamma mangostin [46]. Specifically, study authors have suggested that quercetin, *Ganoderma lucidum*, and mangosteen extract may have properties that downregulate or inhibit cyclooxygenase-2 safely [42–46].

Genoscore to identify polymorphic linked nutrient adjustments

This hypothesis, applies to all genes currently discovered or which will be discovered and any nutritional or dietary supplement ingredient currently available or which will become available.

For example, if a DNA test was measuring two genes through single nucleotide polymorphisms (Gene A and Gene B). The index scores (Genoscore) that would be reported to the clinician and patient would be based upon the number of mutations. An index score of 0 would mean no mutation. An index score of 1 may mean a mutation in Gene A. An Index Score of 2 may mean a mutation in Gene B. An Index Score of 3 may mean a mutation in Gene A and Gene B. It is proposed that utilizing the Genoscore will result in a simple report, easily understandable to both the clinician and patient that provides insights into disease diagnosis, stratification, prognosis, as well as the metabolism, efficacy and/or toxicity associated with specific vitamins, minerals, herbal supplements, homeopathic ingredients and other ingredients in nutritional or dietary supplementation. We hypothesize that utilization of a genetic index score will provide specific information which will ultimately lead to a DNA-based customized nutraceutical (nutritional “gene” therapy) to assist in the diagnosis, stratification, prognosis and treatment for a number of disease states such as the inflammatory process in humans. Furthermore, utilization of this stepwise process will provide a paradigm shift in delaying disease progression and providing symptomatic relief from OA with fewer side effects than ibuprofen [39].

The process

Step 1 of the process involves collection of a DNA sample using a buccal swab, whole blood sample, or other accepted form of collection. Step 2 of the process involves measuring the specific genes using ELISA, TaqMan, PCR, Invader, or other technologies of measuring genetic and/or metabolomic activity, where available. Step 3 involves taking those measurements of genetic mutations and ana-

Table 1 Ingredients that can be adjusted due to genetic mutations

Ingredient	Contributing genes
<i>Ganoderma Lucidum</i>	Ras-Protein and (HLA-DRB1 *0404 and *0101 or PTPN22 R620W)
Gamma-mangostin	Dopamine Receptor D3 Ser9Gly (-205-G/A, -7685-G/C)
Glucosamine sulfate	Glutamine:fructose-6-phosphate amidotransferase (GFPT1 or GFPT 2) variant in exon 14, I471V or 3' UTR, or glucosamine 6-P acetyltransferase
Chondroitin sulfate	Aggrecan proteoglycan allele 27
Folic Acid	MTHFR C677T (heterozygous/homozygous mutant versus homozygous normal)

lyzing them through algorithms to optimize a patient's nutritional supplementation. Step 4 involves selecting the appropriate formulation based upon the genetic index or profile. Here is a basic example to explain a very complicated algorithm when reduced to practice: If Genetic Profile 1, then Formulation 1 which has more of both Ingredients A and B. If Genetic Profile 2, then Formulation 2 which has more of Ingredient A but less of Ingredient B. If Genetic Profile 3, then Formulation 3 which has less of both Ingredient A and B.

In terms of an example of how nutraceutical “gene” therapy could work to treat the signs and symptoms of arthritis, we have presented in Table 1 and Fig. 1:

The exact nutritional supplement formulation will be determined based upon results from a DNA test for the potential joint health product. One important gene, for example may be the genetic mutation in methylene tetrahydrofolate reductase (MTHFR), specifically MTHFR C677T. In light of the higher cardiovascular risks associated with taking over-the-counter or prescription anti-inflammatory drugs, this component of the DNA test can provide additional insights. A mutation of this single nucleotide polymorphism has been clinically found to correlate with:

- Elevated homocysteine (Hcy) levels in the body [47].
- Risk for hypertension [47].
- Cardiovascular disease inflammation markers [48].

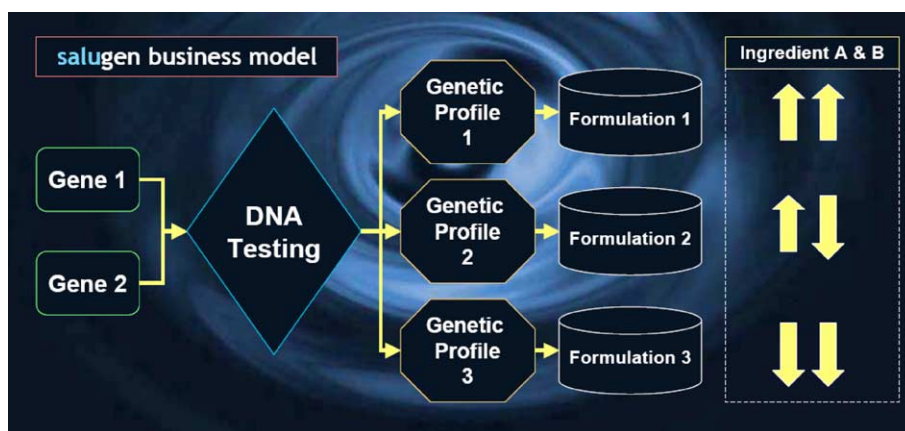


Figure 1 Model of DNA-customized nutritional supplement formulation.

- Prevention of atherosclerosis with tailor-made nutritional supplementation [49].
- Risk for low bone mineral density and osteoporosis [50].
- Incidence of depression [51].
- Risk for stroke [52] and
- Liver toxicity and side effects when taking the most common disease-modifying rheumatoid arthritis medication, methotrexate [53].

758
759 The second genetic mutation that could be mea-
760 sured is Human Leukocyte Antigen DRB1 (HLA-
761 DRB1). This genetic mutation has been associated
762 with the most disabling forms of arthritis – rheu-
763 matoid arthritis – when joints swell and cartilage
764 is damaged. This genetic mutation has been clini-
765 cally found to correlate with

- Risk for Rheumatoid Arthritis [54] and severity of disease progression [55],
- Risk for joint damage from Rheumatoid Arthritis that can be seen on X-rays [56],
- Risk for Rheumatoid Vasculitis [57],

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772 The third gene is the Aggrecan Proteoglycan
773 (AgP). Aggrecan, one of the major structural
774 genes of cartilage, encodes a proteoglycan core
775 protein composed of an extended central glycos-
776 aminoglycan-bearing domain, flanked by globular
777 domains at each end. The central region consists
778 of long stretches of repeating amino acids that
779 serve as attachment sites for glycosaminoglycans
780 such as chondroitin and keratin sulfate; the termi-
781 nal globular domains interact with other cartilage
782 components. The glycosaminoglycan attachment
783 region is encoded in several species by a single
784 large exon, within which are several different
785 types of repeating sequences. Several species
786 show within this exon a similar block of conserved

repeats for attachment of chondroitin sulfate, but
in humans this group of repeats is particularly well
conserved. Examination of genomic DNA from a
population of unrelated individuals by polymerase
chain reaction or Southern blot assays shows this
block of repeat sequences exists in multiple allelic
forms, which differ by the number of repeats at
this site in each allele. Thirteen different alleles
have been identified, with repeat numbers ranging
from 13 to 33. This is an unusual example of an
expressed variable number of tandem repeat poly-
morphism. This polymorphism is apparently re-
stricted to humans, of several species examined.
This polymorphism results in individuals with dif-
fering length aggrecan core proteins, bearing dif-
ferent numbers of potential attachment sites for
chondroitin sulfate. Through analyzing the AgP
gene, the possibility exists for a molecular under-
standing of biological variation in cartilage func-
tional properties [58] which we hypothesize will
logically influence a subject's response to chon-
droitin sulfate and other nutritional supplements
affecting cartilage function.

Summary

Thus, we hypothesize that the combination of
these three genetic mutations, among others, fac-
tored into a proprietary algorithm of laboratory
measures that can be reported as an index score,
will provide patients and clinicians with a genetic
profile offering insights into their healthcare con-
cerns and nutritional needs. Equally as important,
we hypothesize that this genetic index score will
guide the customized nutraceutical formulation
and through this novel detailed genetic approach
result in a paradigm shift to the diagnosis, stratifi-
cation, prognosis and treatment of inflammatory

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823 processes in the human. Furthermore, we have pro-
824 vided support that evidence shows the importance
825 of the dopaminergic connection as an anti-pain and
826 anti-stress molecule, working at the mesocortico-
827 limbic region of the brain, specifically at the NAc.
828 Additionally, we have provided support that clini-
829 cal evidence demonstrates the effectiveness and
830 safety of natural substances for joint health, such
831 as glucosamine sulfate, chondroitin sulfate, *Gano-*
832 *derma lucidum* and HLA that effects Type II colla-
833 gen [59].

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