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The Role of Genomic Oxidative-Reductive Balance as Predictor of Complex Regional Pain Syndrome Development: A Novel Theory

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The aftermath of sequencing the human genome has birthed many efforts to utilize an individual's genetic information in order to tailor optimal treatment strategies — so-called personalized medicine. An individual's genetic information may eventually help diagnosis and treatment, as well selecting optimal pharmacologic agents based partly on how well they reach their target, how well they will bind to and produce an effect at their targets, how well they will be metabolized, and the profile of their adverse effects. It also appears that clinicians may be able to utilize an individual's genetic information to ascertain a subject's risk or susceptibility of developing a particular medical condition. Although, this has not been widely utilized in pain medicine at this point, the future may revolutionize the role of genetic information in the evaluation and management of various pain conditions.

One reason for variations in therapeutic outcomes from different pharmacologic pain treatments is the different genetic disposition of patient to develop pain or to respond to analgesics. The patient's phenotype may represent a conglomerate of several different genetic variants concomitantly present in an individual. Genetic variants may modulate the risk of developing a painful condition, or may modulate the perception of pain (e.g. OPRM1 or GCH1 variants conferring modest "protection" from pain by increasing the tone of the endogenous opioid system or decreasing nitric oxide formation). Other genetic polymorphisms may alter pharmacokinetic mechanisms (e.g. CYP2D6 related prodrug activation of codeine to morphine), alter pharmacodynamic mechanisms (e.g. opioid receptor mutations), or alter other analgesic effects (e.g. diminished euphoric effects from opioids potentially due to DRD2 polymorphisms decreasing the functioning of the dopaminergic reward system).

This article theorizes that genetic alterations including functional polymorphisms of Nrf2 (a master regulator of the transcription of multiple antioxidants) may render certain subjects more or less susceptible to developing complex regional pain syndrome after surgery or trauma. If this hypothesis is correct, knowing this information may translate into significant and "far-reaching" effects on clinical decision-making surrounding the management of pain in patients who may be more susceptible to develop complex regional pain syndrome. Furthermore, it could lead to the development of novel prevention or intervention strategies, in efforts to prevent, abort, or ameliorate the development of and/or effectively treat complex regional pain syndrome.

Key words: Complex regional pain syndrome (CRPS), single nucleotide polymorphism (SNPs), Nrf2, antioxidant, oxidative stress, NQO1, ARE, NF-κB.

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The "nature versus nurture" debate has been going on for many decades. It appears that there is general agreement that both gene-host and gene-environment interactions may play significant roles in contributing to development as well as intensity/expression of various medical conditions. Additionally, there is a growing appreciation of the existence of various genetic functional polymorphisms that may significantly affect changes in crucial protein/enzyme expression resulting in altered clinical outcomes.

The Endogenous Antioxidant System

The endogenous antioxidant system (EAS) consists of a number of proteins or peptides (e.g., enzymes) and small molecules (e.g., vitamins C and E) that maintain the reducing environment of the body (1). Classical antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) directly scavenge reactive oxygen species (ROS) and prevent ROS-initiated reactions (1). Additionally, 2 biologically important small thiol-containing compounds, glutathione (GSH) and thioredoxin (Trx), are involved in antioxidant defense by serving as substrates for antioxidant enzymes such as GPx and Trx peroxidase in redox cycles (1). The ratio of GSH to GSH disulfide (2GSH: GSSG) has been regarded as a parameter of cellular redox status (1). Trx is located in the inner mitochondrial membrane where it scavenges ROS, and is also known to activate mitochondrial antioxidants such as SOD2 (2). Overexpression of Trx or exogenous administration of Trx enhanced protective effects against oxidative stress and inflammation (3,4). Phase 2 detoxifying enzymes contribute to biosynthesis/ recycling of thiols or facilitate excretion of oxidized, reactive secondary metabolites (e.g., quinones, epoxides, aldehydes, peroxides) through reduction/conjugation reactions during xenobiotic detoxification (e.g. glutathione-S-transferase [GST] isozymes, and NADP(H):quinone oxidoreductase [NQO1]). Also, stress response proteins such as heme oxygenase (HO)-1 and heavy (FTH) and light (FTL) chains of ferritin are cytoprotective against various oxidant or pro-oxidant insults (5,6).

SNPs

There exists significant variation in the human genetic material of different individuals. Single nucleotide polymorphisms (SNPs) are exceedingly common polymorphisms and appear to contribute to approximately 90% of genetic variation (7). For every 100 – 300 base pairs in the human genome one SNP seems to occur. This yields roughly over 11 million SNPs which are identified by the National Center for Biotechnology Information (NCBI) SNP database (dbSNP 127) in the human population with over 5 million validated by multiple investigators (8). This should result in approximately 165,000 SNPs within the 20,000 – 25,000 estimated genes whose coding regions vocer approximately 1.5% of the human genome (9,10).

SNPs that affect gene expression occur in all regions of the genome. SNPs located within the coding region of genes, including those that cause amino acid codonalterations (non-synonymous variants) can lead to protein misfolding, polarity shift, improper phosphorylation, and other functional consequences. Variants located within non-coding regions of the genome, while mostly regarded as non-functional, can impact gene regulatory sequences such as promoters, enhancers, and silencers (11) and are termed regulatory SNPs (rSNPs) (12-15). In some cases, a SNP in a transcription factor binding site may increase or decrease the binding between transcription factors and transcription factor binding sites, leading to allele-specific gene expression (8). Thus, functional rSNPs in TF binding sites may predictably lead to differences in gene expression and phenotypes, and ultimately affect susceptibility to environmental exposure (8).

There are multiple instances where genetic variations may potentially modulate pain sensitivity/perception and/or analgesic responsiveness. There are substantial individual differences in human responses to painful stimuli and to opiate drugs some of which are attributed to genetic variations in the mu-opioid receptor (OPRM1). Shabalina et al (16) provide evidence for an essential role for MOR-1K isoforms in nociceptive signaling and suggest that genetic variations in alternative OPRM1 isoforms may contribute to individual differences in opiate responses. Huang and colleagues (17) suggested an association between the IVS2+31G > A SNP of the OPRM1 gene and pressure pain sensitivity in healthy adult females. Ginosar and colleagues (18) demonstrated increased opioid requirements for alfentanil in patients with the A118G SNP, who self-administered a higher dose, achieved higher plasma concentration, and yet complained of more severe pain, suggesting that A118G SNP impairs the analgesic response to opioids. Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor (e.g. altered regulation of protein kinase A [PKA] and ERK½) and may contribute to the genetic risk for addiction. Walter and Lötsch (19) performed a meta-analysis and found no consistent association between OPRM1 118A>G genotypes and most of the phenotypes in a heterogeneous set of 8 clinical studies. This indicates that despite initially promising results, available evidence of the clinical relevance of the OPRM1 118A>G polymorphism is not supported by a meta-analysis (19).

GTP cyclohydrolase (GCH1), recently implicated in shaping pain responses in rodents and humans, regulates production of g(R)-L-erythro-5,6,7,8-tetrahydro-

biopterin (BH4), an essential cofactor for the synthesis of dopamine, serotonin, and nitric oxide (20). Tegeder et al (21) discovered a haplotype associated with reduced ratings of experimental pain stimuli in normal volunteers, and a favorable outcome with regard to long-term pain reduction in patients that underwent lumbar disc surgery. In another study, Tegeder and colleagues (22) showed that carriers of the particular GCH1 haplotype addressed in this study had higher thresholds to punctuate mechanical pain (von Frey hairs) following local skin inflammation (18.1±11.3 vs 9±2.8g; *P*=0.005) and, to a lesser degree to heat pain following capsaicin sensitization (35.2±0.99 vs 36.6±2.4 degrees C; *P*=0.026). However, Kim and Dionn (23) failed to replicate significant associations between the same GCH1 genomic variants and pain responses, both in assessment of experimental pain and in a postoperative third-molar dental pain model. Campbell et al (20) analyzed the association of five previously identified GCH1 SNPs with ratings of pain induced by topical high concentration (10%) capsaicin applied to the skin of 39 healthy human volunteers. Each of the GCH1 polymorphisms was associated with lower pain ratings. When combined, 3 of the 5 accounted for a surprisingly high 35% of the inter-individual variance in pain ratings (20). Campbell and colleagues (20) concluded that SNPs of the GCH1 gene may profoundly affect the ratings of pain induced by capsaicin.

Diatchenko et al (24) suggested that the val(158) met SNP plays a primary role in variation in temporal summation of pain, but that other SNPs of the COMT haplotype may exert a greater influence on resting nociceptive sensitivity. Treister et al (25) studied 30-bp repeat in the promoter region of the monoamine oxidase-A gene (MAO-A), 40-bp repeat in the 3'-intranslated region of the dopamine transporter gene (DAT-1), and 48-bp repeat in the exon 3 of the dopamine receptor 4 gene (DRD4) and found significant associations between cold pain tolerance and DAT-1 (*P*=0.008) and MAO-A (*P*=0.024) polymorphisms; suggesting that low dopaminergic activity can be associated with high pain sensitivity and vice versa.

Furthermore, there are numerous examples of rSNPs associated with disease susceptibility, including hypercholesterolemia (26), hyperbilirubinemia (27, 28), myocardial infarction (29), acute lung injury (30), and asthma (8, 31).

CRPS

Complex regional pain syndrome (CRPS) is one of the most mysterious and challenging pain syndromes facing clinicians. The old label reflex sympathetic dystrophy (RSD) was changed to CRPS in 1994 at a consensus workshop in Orlando, Florida (32,33). The new name and diagnostic criteria was codified by the International Association for the Study of Pain (IASP) task force on taxonomy (Table 1) (34). The new diagnostic entity of CRPS was intended to be descriptive, general, and not imply any specific etiology (including any direct role for the sympathetic nervous system), since the pathophysiology of CRPS remains uncertain (35).

A factor analysis that was conducted in a series of 123 CRPS patients indicated that signs and symptoms of CRPS actually clustered into four statistically distinct subgroups (36). The first of these subgroups is a unique set of signs and symptoms indicating abnormalities in pain processing (e.g., allodynia, hyperalgesia) (35). Skin color and temperature changes, which are indicative of vasomotor dysfunction, characterize the second subgroup (35). Edema and sudomotor dysfunction (e.g., sweating changes) combined to form a third subgroup. A fourth subgroup included motor and trophic signs and symptoms (35). Clinical Diagnostic Criteria were endorsed by an invitation-only workshop which met in Budapest, Hungary in the fall of 2003 (summarized in Table 2).

Although, the mechanisms contributing to CRPS are not known, it is conceivable that ROS may contribute to CRPS pathophysiology. Free radical scavengers, N-acetyl-L-cysteine and 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine, reduce signs of hyperalgesia and allo-

Table 1. *IASP diagnostic criteria for complex regional pain syndrome (CRPS).**

1. The presence of an initiating noxious event, or a cause of immobilization
2. Continuing pain, allodynia, or hyperalgesia in which the pain is disproportionate to any known inciting event
3. Evidence at some time of edema, changes in skin blood flow, or abnormal sudomotor activity in the region of pain (can be a sign or symptom)
4. This diagnosis is excluded by the existence of other conditions that would otherwise account for the degree of pain and dysfunction

* If seen without "major nerve damage" diagnose CRPS I; if seen in the presence of "major nerve damage" diagnose CRPS II.
Not required for diagnosis; 5–10% of patients will not have this.
Adapted from Merskey and Bogduk, 19949 (34)

dynia in animal models of CRPS (37). Clinically, topical treatment with 50% dimethyl sulfoxide cream can be effective in decreasing the hypoxia-related production of free oxygen radicals (38). Coderre et al (39) found that ROS may contribute to CRPS pathophsyiology utilizing an ischemia-reperfusion animal model of CRPS, (chronic post-ischemia pain [CPIP]), however, this model has been criticized as not being an ideal animal model correlate of human CRPS (40).

In a double-blind, prospective, multicenter trial, 416 patients with 427 wrist fractures were randomly allocated to treatment with placebo or treatment with 200, 500, or 1,500 mg of vitamin C daily for 50 days. The effects of gender, age, fracture type, and cast-related complaints on the occurrence of CRPS were analyzed (41). Three hundred and seventeen patients with 328 fractures were randomized to receive vitamin C, and 99 patients with 99 fractures were randomized to receive a placebo. The prevalence of CRPS was 2.4% (8 of 328) in the vitamin C group and 10.1% (10 of 99) in the placebo group (P = 0.002); all of the affected patients were elderly women. Analysis of the different doses of vitamin C showed that the prevalence of CRPS was 4.2% (4 of 96) in the 200-mg group (relative risk, 0.41; 95% CI, 0.13 to 1.27), 1.8% (2 of 114) in the 500-mg group (relative risk, 0.17; 95% CI, 0.04 to 0.77), and 1.7% (2 of 118) in the 1500-mg group (relative risk, 0.17; 95% CI, 0.04 to 0.75). Early cast-related complaints predicted the development of CRPS (relative risk, 5.35; 95% CI, 2.13 to 13.42) (41). Zollinger et al concluded that vitamin C reduces the prevalence of CRPS after wrist fractures. A daily dose of 500 mg for 50 days is recommended (41).

CRPS and Oxidative Stress

Eisenberg and his colleagues (42) demonstrated very large increases in malondialdehyde, lactic dehydrogenase, and cellular antioxidants (peroxidase, superoxide dismutase, and uric acid) in the serum, and especially in the saliva, of 31 CRPS-I patients versus 21 healthy volunteers. Malondialdehyde (MDA) is produced when the phospholipids of cell membranes are damaged by reactive oxygen species; it is a widely accepted marker for oxidative stress. Serum lipid peroxidation products (MDA) and all antioxidative parameters analyzed were significantly elevated in CRPS-I patients: median salivary peroxidase and superoxide dismutase (SOD) activity values, uric acid (UA) concentration, and total antioxidant status (TAS) values were higher in CRPS-I patients by 150% ($P = 0.01$), 280% ($P = 0.04$), 60% ($P = 0.0001$), and 200% ($P = 0.0003$), respectively, as compared with controls (42). Lactic dehydrogenase levels are also known to increase in the presence of oxidative stress. Median salivary albumin concentration and median salivary LDH activities in the patients were 2.5 times ($P = 0.001$) and 3.1 ($P = 0.004$) times higher than in the controls (42). The collective data support the concept that free

Table 2. *Proposed clinical diagnostic criteria for CRPS.*

General definition of the syndrome: CRPS describes an array of painful conditions that are characterized by a continuing (spontaneous and/or evoked) regional pain that is seemingly disproportionate in time or degree to the usual course of any known trauma or other lesion. The pain is regional (not in a specific nerve territory or dermatome) and usually has a distal predominance of abnormal sensory, motor, sudomotor, vasomotor, and/or trophic findings. The syndrome shows variable progression over time.
To make the *clinical* diagnosis, the following criteria must be met: 1. Continuing pain, which is disproportionate to any inciting event 2. Must report at least one symptom in 3 of the 4 following categories: **Sensory:** Reports of hyperesthesia and/or allodynia **Vasomotor:** Reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry **Sudomotor/Edema:** Reports of edema and/or sweating changes and/or sweating asymmetry **Motor/Trophic:** Reports of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin) 3. Must display at least one sign **at time of evaluation** in 2 or more of the following categories: **Sensory:** Evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch and/or temperature sensation and/or deep somatic pressure and/or joint movement) **Vasomotor:** Evidence of temperature asymmetry (>1°C) and/or skin color changes and/or asymmetry **Sudomotor/Edema:** Evidence of edema and/or sweating changes and/or sweating asymmetry **Motor/Trophic:** Evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin) 4. There is no other diagnosis that better explains the signs and symptoms
For *research* purposes, diagnostic decision rule should be at least one symptom ***in all 4*** symptom categories and at least one sign (observed at evaluation) in 2 or more sign categories (ref 35).

radicals are involved in the pathophysiology of CRPS-I, which is reflected both in serum and salivary analyses (42).

The increased levels of endogenous antioxidants almost certainly represent a compensatory response to the oxidative stress. Nitric oxide levels in fluids aspirated from artificial suction blisters, which occurred in the affected limb of patients with CRPS-I were significantly lower, as compared to blisters in the contralateral healthy limb (43). Free radicals, in turn, can increase vascular permeability, release neuropeptides (i.e. substance *P*), enhance inflammation, and cause further tissue damage (44,45). Multiple clinical trials have shown that the free radical scavengers, dimethylsulfoxide (DMSO), N-acetylcysteine (NAC), vitamin C, and mannitol, can reduce signs and symptoms of CRPS-I (38,46-50). Taken together, Coderre and Bennett (51) concluded that these data provide unequivocal evidence that oxidative stress is present in CRPS-I patients. The data confirm a large amount of indirect evidence for the presence of oxidative stress in CRPS-I patients and strengthen the rationale for the use of antioxidants and free radical scavengers in the treatment and prevention of CRPS-I (51).

Coderre et al (39) have proposed that at least some CRPS-I patients may have ongoing chronic, deep tissue inflammation due to microvascular pathology caused by ischemia–reperfusion injury. Their hypothesis comes from the work on an animal model of CRPS-I, chronic postischemic pain (CPIP) (39), where an ischemia–reperfusion (I-R) injury to the hind paw is produced by placing a tourniquet around the anesthetized animal's ankle for 3 hours. Prolonged ischemia leads to the accumulation of oxidases, enzymes that produce free radicals (39). The return of oxygenated blood upon reperfusion results in the production of a burst of free radicals (superoxide, hydrogen peroxide, hydroxyl radical, perhydroxyl radical, singlet oxygen, and peroxynitrite anion) that causes an I-R injury to the endothelial cells of the microvasculature (i.e., the capillaries, arterioles, and venules) (39).

Coderre and colleagues (52) provided evidence for the slow-flow/no-reflow effect in the hind paw muscles of CPIP rats and for the I-R evoked arterial hypersensitivity to norepinephrine (similar to CRPS) (53-54). Additionally, they have detected elevated levels of malondialdehyde in the hind paw muscles (52) and have shown that the animal's pain hypersensitivity is reduced by free radical scavengers and antioxidant therapy (39).

Additional Support for the Antioxidant Vitamin C Having Therapeutic Utility in CRPS

A 5-year-old female with clinical and radiographic evidence of scurvy developed features of CRPS 2 years after a left ankle fracture which improved with the administration of vitamin C (55).

Zollinger and colleagues (56) performed 32 arthroplasties for first carpometacarpal arthritis in 27 patients using a cementless total trapeziometacarpal joint prosthesis; a surgery that may be complicated by CRPS. In all their patients Vitamin C 500 mg daily was started 2 days before surgery and continued for 50 days. There were no cases of CRPS under vitamin C prophylaxis (56).

Besse et al (57) studied all patients (except patients with diabetes mellitus) having surgery on the foot or ankle in 2 groups, without (Group I) and with (Group II) 1 gm daily of preventative oral vitamin C treatment. 420 feet (from 392 patients) were included in the study: 185 in Group 1, and 235 in Group II. CRPS I occurred in 18 cases in Group I (9.6%) and 4 cases in Group II (1.7%) (P <0.001) (57). Besse and colleagues (57) concluded that vitamin C is effective in preventing CRPS I of the foot and ankle since it was not an infrequent complication in their control group (9.6%).

Nrf2

Nuclear factor-erythroid 2 related factor 2 (Nrf2) is an ubiquitous master transcription factor that regulates antioxidant response element (ARE)-mediated expression of antioxidant enzyme and cytoprotective proteins (58, 59). Nrf2 belongs to the "cap'n collar" (CNC)-basic region/leucine zipper (bZIP) transcription factor family (58, 59). In the cytoplasm of unstressed cells, Kelch-like ECH associating protein 1 (Keap1) is a cytoplasmic cysteine rich, actin bound protein that sequesters Nrf2 from activating factors by binding to the N-terminal Neh2 domain (59). Oxidative stress leads to activation of Nrf2 by phosphorylational mediation via several protein kinase pathways resulting in Keap1· Nrf2 dissociation, Nrf2 nuclear translocation, and binding of Nrf2 to the cis-acting ARE (60).

Nrf2 must form a heterodimer with other bZIP transcription factors such as small Maf (58), c-Jun (61), activating transcription factor (ATF)-4 (62), c-Fos, or Fra-1 (63) for ARE binding and target gene expression. Coactivator proteins CREB binding protein (CBP)/p300 and ARE-binding protein-1 are presumed to interact with Nrf2·Maf to regulate ARE-dependent gene expression (64). Certain small Maf genes (e.g., mafG, mafF) ap-

pear to be essential for the activation of antioxidant response element-dependent genes (65). Katsuoka and colleagues (66) demonstrated that mafG is itself an ARE-dependent gene that is regulated by an Nrf2/small Maf heterodimer and suggested the presence of an auto-regulatory feedback pathway for mafG transcriptional regulation. Katsuoka et al (65) developed triple-mutant fibroblasts that completely lack small Mafs which were highly susceptible to oxidate stress.

The Nrf2·Maf complex binds to the consensus sequence 5_GTG-ACNNNGC-3′ (known as aARE) to induce transcription of ARE-bearing detoxifying enzymes such as NAD(P)H:quinone oxidoreductase, certain glutathione-S-transferases, and γ-glutamate cysteine ligase regulatory subunit, as well as classical antioxidant enzymes (e.g., catalase and superoxide dismutase) and heme oxygenase-1 (67,68). NRF2, thus, appears to be an essential regulatory element in response to oxidant injury (69).

Bach 1, the negative regulator of Nrf2, competes with Nrf2 for binding to the ARE in the human NQO1 promoter. Antioxidants induced phosphorylation of tyrosine486 which leads to rapid nuclear export of Bach1 that slows Nrf2 access to bind to ARE and activate/upregulate defensive genes that protect cells against oxidative and eletrophilic stress (70). Leucine zipper transcription factor 1 is a transcription repressor that is conserved and ubiquitously expressed in tissues (71-74). In the absence of cellular stress, Bach1 heterodimers with small Maf proteins that bind to the ARE repressing defensive gene expression (71,72,75). There appears to be a competetitive interplay between the Bach1-containing repressor dimers and Nrf2-containing activator dimers (72,76).

Stress inducing agents that activate antioxidant enzyme expression failed to further enhance Nrf2 mRNA levels above the basal level; therefore the data of Marzec et al (69) are consistent with the contention that the -617 and -651 SNPs affect the basal Nrf2 message levels. Formation of protein-DNA complex was significantly diminished in heterozygotes ($P < 0.001$) and variants ($P < 0.001$) for the -617 polymorphism in the ARE-like sequence (69). The significant reduction of protein-DNA complex indicates that the -617 SNP affects efficient binding of proteins, such as Nrf2, to the ARE-like site (69). Thus, Nrf2 binds less efficiently to ARE-like sequences that contain the -617 polymorphism, as compared with the wild-type allele. Supershift assays with anti- Nrf2 antiserum revealed binding of Nrf2 to the wild-type ARE-like sequence, suggesting that Nrf2 autoregulates transcription through this promoter region (69). In support of this notion, Kwak et al (77) showed that Nrf2 binds to the ARE-like element of the mouse Nrf2 promoter (-754) and up-regulates its transcription (69).

Marzec et al (69) identified 6 novel SNPs in Nrf2. Three promoter polymorphisms were predicted to have functional significance, and one (-617 [C/A]) significantly affects basal NRF2 expression and function. These polymorphisms were also found in a Japanese population by Yamamoto et al (78). The human -617 SNP was predicted to affect Nrf2 ARE-like promoter binding sites (69).

Marzec and colleagues (69) found that the -617 SNP was associated with an increased risk of developing acute lung injury (ALI) in a nested case-control study of at-risk patients with major trauma, suggesting a role for Nrf2 in development of the syndrome. The mechanism through which Nrf2 confers protection against oxidative stress likely relates to the ability of this transcription factor to regulate antioxidant and phase II enzyme genes that bear promoter AREs in their regulatory (promoter and/or enhancer) regions (79-81). It may be postulated that individuals with functional polymorphisms in Nrf2 that alter basal expression of Nrf2, or the ability of Nrf2 to translocate from the cytoplasm to nuclear binding sites, are at enhanced risk of oxidative stress and ALI. Consistent with this hypothesis, targeted disruption of Nrf2 significantly decreased antioxidant capacity in mice, and thus enhanced susceptibility to prooxidant, -fibrotic, and -carcinogenic agents (82-84). Diminished or dysfunctional Nrf2 may not only be involved in lung dysfunction but potentially may be involved in other pathophysiologic conditions. Impairment of Nrf2 activity may represent a major risk factor for the evolution of non-alcoholic steatohepatitis (NAFLD) or non-alcoholic fatty liver disease (NASH) (85).

Considering the role of Nrf2 in the protection against oxidative states and the potential benefit of antioxidants in the development and maintenance of CRPS it is conceivable that functional polymorphism of this transcription factor may be one of several alterations affecting susceptibility to CRPS.

Regulation of the function of KEAP1 may affect overall Nrf2 activity and/or over-expression of Bach1 may antagonize Nrf2 activity leading to repression of oxidants and thus affect susceptibility to CRPS. Other SNPs could also interact with previously discussed SNPs or separately affect susceptibility to CRPS. Alternatively, it is conceivable that there may be SNPs that exist which

could contribute to diminishing one's risk of developing CRPS.

Reddy and colleagues found that the A/C SNP at -1221decreased in vitro transcription of NQO1 at baseline and after exposure to hyperoxia and other oxidant stressors. Patients heterozygous for the -1221 C allele were at significantly lesser risk of ALI after major trauma compared with patients with wild type alleles, even after adjustment for APACHE III score, and mechanism of trauma (OR, 0.46 [95% CI, 0.23, 0.90]; $P = 0.024$) (86). Reddy et al (86) characterized functional promoter SNPs in the phase II antioxidant gene NQO1 (NAD(P)H:quinone oxidoreductase1) to evaluate its role in susceptibility to ALI. Thus, it may be possible that subjects with the AC genotype at position -1221 in the NQO1 gene may be less susceptible to developing CRPS then the general population.

CRPS and NF-κB

Nuclear factor kappa B (NF-κB) has multiple similarities to Nrf2, being a master transcription factor which usually regulates genes predominately related to innate immunity, inflammation, and apoptosis. Also, like Nrf2, it is normally "sequestered" in the cytoplasm in an inactive state bound to inhibitors of NF-κB (IκB). IκB is analgous to Keap1. IKK may then phosphorylate IκB leading to dissociation of NF-κB and IκB.

NF-κB is involved in several pathogenic mechanisms that are believed to underlie the CRPS, including ischemia, inflammation and sensitization (87). Chronic postischemia pain (CPIP) has been developed as an animal model that mimics the symptoms of CRPS-I (87). de Mos et al (84) studied the possible involvement of NF-κB in CRPS-I using CPIP rats (with 3 hours of ischemia, followed by rapid reperfusion [IR injury]). The NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) was administered systemically, intrathecally, and intraplantar injection to evaluate its effects on mechanical/thermal allodynia. At 2 and 48 hours after IR injury, NF-κB was elevated in muscle and spinal cord of CPIP rats compared to shams (87). At 7 days, NF-κB levels were normalized in muscle, but still elevated in spinal cord tissue. Systemic PDTC treatment relieved mechanical and cold allodynia in a dose-dependent manner, lasting for at least 3 hours (87). Intrathecal — but not intraplantar administration also relieved mechanical allodynia. de Mos and colleagues (87) delivered their results suggested that muscle and spinal NF-κB plays a role in the pathogenesis of CPIP and potentially of human CRPS.

CRPS AND Nrf2

Currently there is no evidence directly linking Nrf2 to CRPS pathophysiology, however since Nrf2 is a major transcription factor regulating multiple endogenous antioxidants, the author feels that it is somewhat intuitive that suboptimal Nrf2 activity may be involved in certain cases of CRPS.

CRPS and SNPs

The author proposes that there may exist SNPs such as the -617 polymorphism or other functional polymorphisms of Nrf2 which would result in reduced quantity and/or quality of antioxidant activity, which could potentially lead to significant clinical consequences. A patient with such a functional polymorphism may exhibit Nrf2 activity resulting in an impaired endogenous antioxidant "defense" system. Such a patient would be theoretically more susceptible to oxidative stress and thus, potentially more susceptible to develop CRPS (Fig. 1).

Summary

Based on the above discussion, the author proposes that Nrf2 SNPs may occur in a subpopulation of patients with CRPS and that the occurrences of these SNPs may contribute to factors that may make these patients more susceptible to develop CRPS than the general population. CRPS is a complex disorder and thus, a SNP association could be due to the effects of other polymorphisms, both within Nrf2 and at nearby loci. Marzec et al (69) identified functional polymorphisms in the promoter of Nrf2 that are found in relatively high frequency among multiple ethnic populations (69). In a nested case-control study, patients with the –617 A SNP had a significantly higher risk for developing ALI after major trauma (OR 6.44; 95% CI 1.34, 30.8; $P = 0.021$) relative to patients with the wild type (–617 CC).

The author proposed that the -617 variant and/or other functional polymorphisms may be instrumental in contributing to suboptimal Nrf2 activity and thus to patients that may represent humans who are at risk or susceptible to develop complex regional pain syndrome. If confirmed, this information could be clinically important, as it could enable patients and health care providers to help make more informed clinical decisions affecting lifestyle and potential therapeutic strategies.

For example, if the author's theory is correct and the patient's -617 variant status is known for a particular patient: that patient may choose not to pursue a

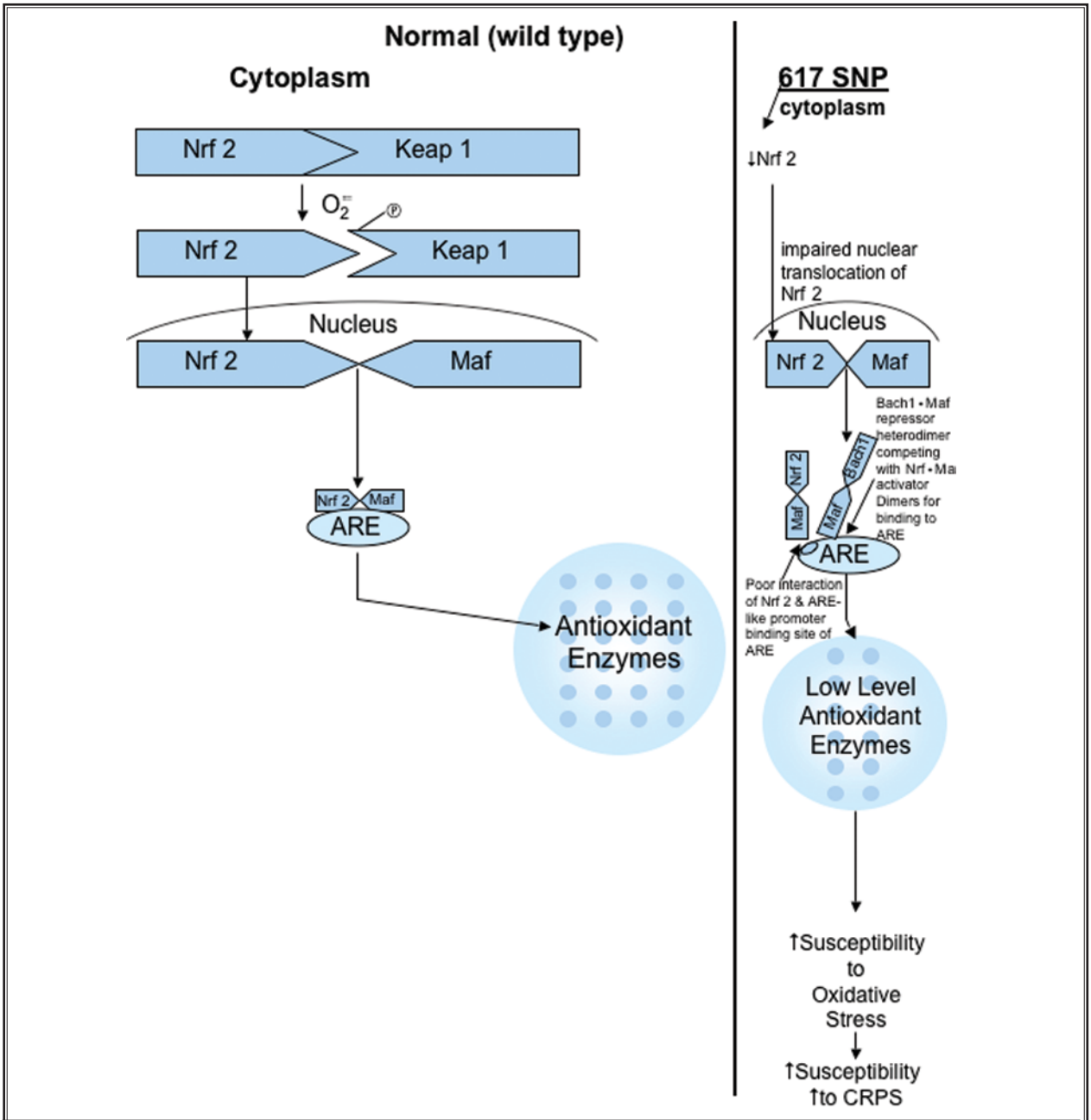

Fig. 1. *Hypothetical schematic of potential effects of functional polymorphism on susceptibility to CRPS.*

career as a stuntman/stuntwoman where they may be subjected to repeated "potentially triggering" trauma, or choose to go on lifelong antioxidant therapy. Additionally, clinicians may decide to aggressively treat these patients extremely early — at the first sign of any symptoms/trauma. Also, clinicians may decide to perform a "prophylactic"/pre-emptive preoperative sympathetic block for such a patient undergoing surgery on an extremity (although the article which showed this technique to be potentially beneficial in patients undergoing surgery with a history of CRPS may have had falsified data) (88).

Contrariwise, subjects who possess the AC genotype at position -1221 in the NQO1 gene with decreased transcription of NQO1 may potentially be less susceptible to the development of CRPS and perhaps could be observed by clinicians or treated by nonagressive means initially upon the development of CRPS-like symptoms. The author encourages future translational investigations into the role of -617 variant and various other functional polymorphisms contributing to suboptimal Nrf2 activity and increasing susceptibility for developing CRPS as well as roles in other oxidant-related conditions or disease processes. These investigations could conceivably lead to the development of novel prevention or intervention strategies for CRPS.

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