



## Nutritional, metabolic and genetic considerations to optimise regenerative medicine outcome for knee osteoarthritis



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### ABSTRACT

Knee osteoarthritis (KOA) is a multifactorial degenerative disorder of joints, affecting the world's population over the age of 65 and with a higher prevalence in females. KOA is responsible for many age associated joint problems such as stiffness and pain. Conventional methods for managing KOA such as nonsteroidal anti-inflammatory drugs (NSAID) may not improve pain or alter the disease progression and may have adverse side effects. Non-pharmacological management of OA is fundamental to management of functional limitations and provides effective symptom relief but has not shown that disease progression can be altered. Regenerative medicine is a relatively new approach which aims to induce cellular regeneration and promote self-healing through minimally invasive methods. The use of regenerative medicine slowed the progression of KOA and revealed significant improvements, yet further investigations are required to optimize the outcomes. Nutritional and metabolic aspects such as supplementations, vitamins and minerals were proven to have an impact on the progression of KOA. Genetic variations are rapidly inspected to identify any potential influence of these variations in the predisposition and diagnosis of KOA. Further supporting evidence suggests the potential influence of metabolic, nutritional and genetic aspects in optimizing the outcomes of regenerative medicine in the management of KOA.

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### 1. Introduction

Osteoarthritis (OA) is the most common chronic joint disorder and the main cause of joint pain, loss of function and disability in adults. As reported in research OA is prevalent in individuals above the age of 65 years old.<sup>1</sup> 80% of the population over the age of 75 years suffers from Knee osteoarthritis (KOA).<sup>1,2</sup> KOA is a degenerative inflammatory disease disturbing various components of the knee and hypertrophy of the joint capsule such as the articular cartilage, synovial joint lining, periarticular bone and adjacent supporting fibrocartilaginous and musculature structures.<sup>1,3</sup> Augmented concentrations of activated proteins and cytokines is reported with the progressive loss in the articular cartilage,

subchondral bone and chondrocytes.<sup>1,4</sup> The diagnosis of KOA utilise a combination of anatomic analysis and imaging to identify KOA-induced structural damage and classify the patient's condition to mild, moderate or severe KOA accordingly.<sup>4</sup>

KOA is a multifactorial disease. Intrinsic and extrinsic factors interact and contribute at various levels to the evolution of this multifactorial disease (Fig. 1). The pathogenesis of KOA is influenced by several genetic factors as well as environmental factors related to molecular pathways that contribute to articular injury.<sup>5</sup> While the knowledge of the main cause of KOA is insufficient, age, sex and injury's link to KOA has been established among ethnicities.<sup>3</sup>

Chronic disease such as KOA, is progressive and is preceded by a period of declining function in one or more of the biological systems. Restoring health requires improving specific dysfunctions that have contributed to the disease state. Conventional treatments have only showed limited clinical benefits. Current pharmacological treatments such as nonsteroidal anti-inflammatory drugs

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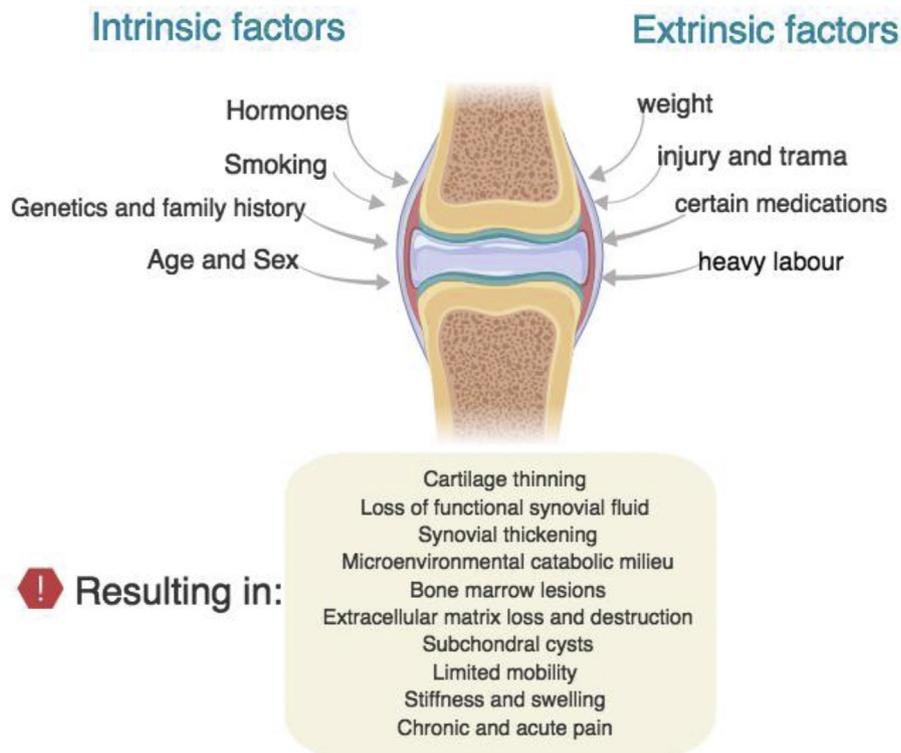


Fig. 1. Factors contributing to the progression of KOA.

(NSAID) show negligible efficacy in disease modification, have adverse side effects and may not improve pain relieve or alter the disease progression in KOA.<sup>6</sup> Moreover, pain management medications carry most of the financial burden in OA.<sup>7</sup> Innovative regenerative engineering strategies such as cell-based therapies are currently in development and have shown early promise in both preclinical and clinical studies. Vega et al. has reported that autologous bone marrow derived mesenchymal stromal cells (BMSC) treatment for knee OA are safe and robust in clinical efficacy.<sup>8</sup>

There is a substantial need for new and emerging interventions for KOA for prevention and modification of the disease process. This review will discuss current evidence of vitamins, minerals, hormones, genetics and their potential role in the progression and management of KOA. We aim to identify the modifications and considerations required in this aspect to improve the results of intra articular injections for regenerative medicine.

## 2. Materials and methods

A systematic search was conducted in PubMed and Web of Science of relevant articles in English through June 2018. A total of 548 articles were retrieved and screened in respect to the inclusion and exclusion criteria in Table 1. Cohort and case-controlled studies will be included for the analysis. All references used were evaluated according to their level of evidence, levels 1,2,3 and 4 were included. The accepted studies are 29 articles as shown in Fig. 2.

## 3. Functional medicine and nutrition

Functional medicine is a dynamic approach to evaluating, preventing and treating complex chronic diseases as it relates to the interconnections of our biological systems.<sup>9</sup> Dysfunctions in our

body may result from life-long interactions with our environment, lifestyle, genetics and predispositions.<sup>9</sup>

### 3.1. Reactive oxygen species (ROS) and inflammation

The etiology of KOA is multifactorial and includes oxidative stress (OXS) and overproduction of reactive oxygen species (ROS). ROSs regulate intracellular signalling processes, chondrocyte degradation and apoptosis, extracellular matrix synthesis and degradation along with synovial inflammation and dysfunction of subchondral bone.<sup>5</sup> Inflammation and its cascading effects has shown to directly affect synovial cells and chondrocytes within cartilage resulting in a release of cytokines such as interleukin-1- $\beta$  (IL-1 $\beta$ ) and to increase the production of metabolic proteins such as proteinases.<sup>10,11</sup> The imbalance ROS results in increased OXS production which evidence demonstrates its contribution to OA. A study by Suantawee et al., found patients with KOA had higher levels of plasma OXS parameters and lower levels of plasma antioxidant parameters than those of healthy controls.<sup>10</sup>

### 3.2. Supplements

Malek et al., evaluated the effects of L-carnitine supplementation on OXS lipid profile and clinical status in women with KOA.<sup>12</sup> The authors found improvement in clinical status and no differences in serum OXS parameters and lipid profile between the L-carnitine and placebo groups.<sup>12</sup> A later study by Malek et al. found L-carnitine to exhibit anti-inflammatory effects by decreasing serum inflammatory mediators, IL-1 $\beta$  and MMP-1 and reducing reported pain in KOA.<sup>13</sup>

Nutraceuticals are considered dietary supplements from natural sources, and they are used as an alternative to pain killers,

**Table 1**  
Eligibility criteria for literature search.

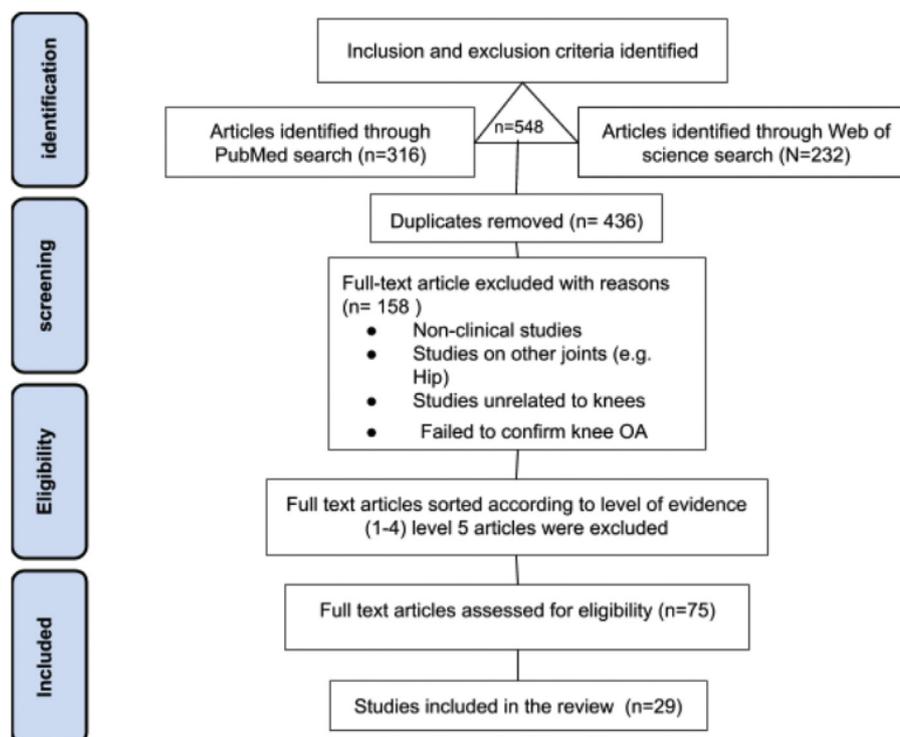
Criteria	Inclusion Criteria	Exclusion Criteria
Level of evidence	Level of evidence 1–4 original articles assess nutritional, metabolic and genetic studies on knee OA	Level of evidence 5 articles, unpublished material (PhD/MSc thesis), letters to the editor, reviews and conference abstracts.
Subjects	Human subject aged $\geq 18$ with primary or secondary confirmed knee osteoarthritis.	pilot studies ( $n < 20$ human participants), animal, cadaver studies.
Study design	Clinical trials, meta-analysis.	Studies assess the treatment or diagnostic method on joints/regions other than knee OA, with exception of heterogeneous studies reporting knee OA results separately. Drug trials.
Study outcome	Studies assess the diagnostic and treatment methods Studies assess the outcome by approaches that utilise nutrition and genetic test.	Studies on outcome obtained by methods other than nutritional, metabolic and genetic assessments (imaging findings, biomechanical or microcirculatory outcomes).
Study language	Articles in English only.	Articles in languages other than English.

nevertheless, growing evidence suggests its pronounced potential as a treatment and management tool for musculoskeletal disorders such as KOA. Theracurmin (bio-available curcumin), is another safe and promising natural anti-inflammatory.<sup>14</sup> The results of a 2014 Randomised controlled trial suggest Theracurmin may decrease the pain and discomfort of KOA and improve the patient's general condition and quality of life.<sup>14</sup> The improvements in KOA patients following the use of Theracurmin suggests its potential benefit in supporting regenerative medicine treatment. Vaishya et al., the impact of various nutraceuticals in the management of KOA.<sup>15</sup> Studies show the anti-inflammatory effect of Boswellia in KOA patient through decreasing the production of inflammatory MMPs, and report a generalized decrease in inflammation levels, which supports regenerative medicine applications. Active individuals with increased risk of developing KOA due to wear and tear are advised to supplement with Glucosamine sulfate (GS) due to its properties in supporting bone health. GS contributes to the synthesis of chondrocytes, studies show its epigenetic impact of inflammatory markers.<sup>15</sup>

Although various nutraceuticals have demonstrated to effectively reduce inflammation, oxidative stress and catabolic activity, their effects on disease modification have not yet been clearly demonstrated or are still under investigation. Further high-quality research is needed to evaluate clinical effects of nutraceuticals when used prior and/or post regenerative medicine treatments, and whether the effects change when used concurrently with regenerative techniques, or if nutraceuticals are better serving as complementary medicine for the management of KOA.

### 3.3. Minerals

The potential role of minerals such as magnesium, calcium and zinc is under investigations for their contributions to the management of KOA. Approximately half of the US population has been shown to consume less than the daily requirement of magnesium from foods.<sup>16</sup> Shmagel et al., stated the role of magnesium in pain sensitization in radiographic confirmed KOA, and it's correspondence to elevated serum levels of C-reactive protein.<sup>17</sup> The study



**Fig. 2.** PRISMA systematic literature search inclusion and exclusion criteria.

noted subjects with a low magnesium intake from dietary components and supplements had higher KOA pain and function scores over a 4-year follow up.<sup>17</sup> In the previous study, 68% and 44% of participants, men and women respectively, were below the estimated average requirement, with an average of 309.9 mg/day total magnesium intake in KOA men, and 287.9 mg/day in KOA women.<sup>17</sup>

Calcium and Zinc may play important role in the management of KOA. Calcium aids in keeping osmotic pressure of joint fluid and supplying nutrients to the knee.<sup>18</sup> Xin et al. found that concentration of zinc and calcium in subjects with KOA was higher than that in healthy individuals and may suggest to be an earlier marker in the diagnosis KOA.<sup>18,19</sup> Studies showed that the increased expression of MMP-1, MMP-2, and MMP-9 proteins might be associated with the pathogenesis of KOA, as MMP activity is regulated by calcium and both zinc and calcium are dependent proteinases.<sup>19</sup>

### 3.4. Vitamins

Although the antioxidant properties of vitamin C and E may have a role in protecting joints from the development and progression of KOA.<sup>20</sup> Chaganti et al., found vitamin C and E did not provide protection against KOA, and vitamin C supplementation increased the risk of KOA.<sup>21</sup> This remains consistent with previous animal studies and may be due in part through the local production of TGF- $\beta$ , which was found to be actively expressed in the osteophytes when subjects were given higher doses of vitamin C.<sup>21,22</sup> Further research is needed for the full spectrum of other antioxidant and its effects on KOA.

Vitamin D may play a significant role in the management of KOA. Recent evidence demonstrated that sufficient vitamin D supplementation over a 2 year period decreased loss of tibial cartilage volume, lessened increase in effusion-synovitis volume, and improved physical function,<sup>23</sup> suggesting its preventative role in KOA.

### 3.5. Hormones

Sex differences in OA suggest hormones or alterations in reproductive hormones concentrations may influence the development of OA.<sup>24,25</sup> Compelling evidence in the literature indicates that suboptimal concentrations of steroid hormones can negatively impact bone health, making it more susceptible to physical injury, especially when the hormone in question is estrogen, explaining the higher frequency of KOA in post-menopausal females.<sup>24,26</sup> Ageing is associated with loss of sex hormones in both men and women rendering a joint environment that is more susceptible to damage and degradation.<sup>9,27</sup> Progesterone is the precursor for all steroid hormones and its receptors have been localized in the chondrocytes of knee cartilage.<sup>27</sup> Some studies even show that this hormone stimulates proteoglycan changes in cartilage, either directly or indirectly through cytokines and, identification of estrogen receptors ER $\alpha$  and ER $\beta$  on human articular chondrocytes confirm that cartilage is sensitive to estrogen.<sup>25</sup> Scarcity of this steroid hormone leads to increased osteoclast recruitment and, therefore, enhanced bone resorption as well as impaired mechanosensitivity and mechanotransduction, compromising the optimal level of osteoblast activity in bone deposition. Progesterone has numerous functions including downregulation of the estrogen receptor, inhibition of oestrogen transcription at the DNA level, and local oestrogen metabolism,<sup>9</sup> and may play a role in maintenance of cartilage volume by suppressing the production of MMPs.<sup>18</sup> A study by Sowers et al., found that lower circulating levels of estradiol and estrone metabolites, especially 2-hydroxyestrone, are related to the development of OA.<sup>24</sup> The authors proposed the action of higher 2-hydroxyestrone concentrations in delaying the development of

KOA may be through the arachidonic acid pathway, including the inhibition of leukotriene synthesis and modulation of lipooxygenases.<sup>24</sup> A study by Jin et al., found that progesterone was positively associated with cartilage volume for females, however, it is important to note that the participants in the study were vitamin D insufficient.<sup>27</sup> As KOA is more common in postmenopausal females, limited investigations were performed to study the effect of male hormones on the development of KOA in male.<sup>27</sup> Cicuttini et al., studied the effect of serum testosterone in 45 males, and proposed a contribution of the serum testosterone to the tibial cartilage volume.<sup>28</sup> Yet, further studies are required to confirm the significance of the contribution of serum testosterone to the progression of KOA in males, prior to studying the effect of testosterone levels on the success of regenerative medicine treatments in KOA male patients.

In summary, the lack of homeostasis and the catabolic environment in patients with KOA may be related to higher levels of OXS and inflammation. Vitamins and minerals may play a role in the management of KOA. KOA is more prevalent in females than men and the differences may be suggestive that altered sex hormones may be associated in the pathogenesis in KOA. The combination of impaired mechanosensitivity and estrogen deficiency might culminate in structural weakness and bone fragility. These findings would then suggest that estrogen-deficient patients may very well experience a reduced effectiveness of mechanic stimulus overall as osteoblast activity would then become limited.

## 4. Genetics aspects

Genetics play a critical role in the pathophysiology of knee OA, as it being a complex multifactorial degenerative disorder, genes and leading signalling pathways which are involved in bone, cartilage and ECM formation are frequently investigated for their association with KOA. Genome wide association studies (GWAS), genetic linkage studies as well as twin studies are used to study the impact of genetic vs environmental factors on knee OA.

### 4.1. Genes associated with the predisposition to KOA

T cells are one of the key players in the proinflammatory environment of KOA. Studies identified genetic variations which have a potential impact on the susceptibility and progression of KOA due to their relationship with T cell activity. Butyrophilin-like 2 gene (BTNL2), human leukocyte antigen (HLA I) and Cluster of differentiation protein (CD40) play a role in the inhibition, activation and differentiation of T cells respectively.<sup>29</sup> HLA I is an immune related gene, which encodes for the major histocompatibility complex (MHC I), a key cell-surface protein in innate immunity. GWAS suggested the associated of variations in the BTNL2 rs10947262 SNP and HLA rs7775228 SNP with KOA in the Japanese population.<sup>30</sup> Studies performed on other ethnicities failed to show the association of rs10947262 and rs7775228 with KOA. This insignificance could be due to the linkage disequilibrium common in the HLA region.<sup>29</sup> Yet, Nakajima et al. argue the SNPs investigated occur at a close approximation, and supporting the evidence of the SNPs association with KOA,<sup>30</sup> suggesting further investigations on this region using a twin study, or further investigation on the impact of T cells in the survival of regenerative medication. Once contribution to KOA is identified, it can be confirmed by studying tagged chondrocytes and their action in activating the T cell number.

Deng et al. studied the association of the CD40 gene polymorphism to the susceptibility and severity of KOA and identified the SNPs rs4810485 and rs1883832 as genes linked with severe KOA genes in the Chinese population.<sup>31</sup>

Tumour necrosis factor (TNF $\alpha$ ) is a proinflammatory cytokine,

which further activates and renews other inflammatory mediators such as IL1. The variation rs1800629 is associated with increased risk of KOA in Asian and Caucasian ethnicities.<sup>32</sup> No functional studies clearly explain the relation between TNF $\alpha$  rs1800629 SNP and the cellular pathogenesis of KOA, but the increased circulating rs1800629 TNF $\alpha$  mRNA could be responsible for the observed associated increased stiffness in Chinese KOA patients, due to the aggravated degeneration action of TNF $\alpha$  on cartilage ECM integrity through the degradation of collagen and proteoglycans.<sup>33</sup> The association between AA genotype and the increased expression of TNF $\alpha$  couldn't be confirmed due to the contradicting results of the major meta-analysis performed on rs1800629.<sup>32,34</sup>

As discussed earlier, woman and specifically post-menopausal woman are more prone to OA including KOA.<sup>25</sup> Researchers suggest that gender-specific hormones are behind the higher prevalence in woman to KOA compared to men.<sup>26</sup> Dai et al. confirmed the SNP rs2234693 in estrogen receptor alpha gene (ESR1) is significantly higher in female KOA patients,<sup>35</sup> which suggests further functional genetic studies to discover the role of the rs2234693 in the degeneration of chondrocytes carrying the ESR1 in KOA.<sup>36</sup> Replication of the GWAS study on a bigger population is required as KOA female subjects had significantly higher BMI than control subjects.<sup>35</sup>

#### 4.2. Genes associated with the pathogenesis of KOA

A disintegrating and metalloprotease 12 (ADAM 12) gene coding protein of the ADAM protein family of the ADAM family involved in adhesion and intracellular signalling. The genetic impact of ADAM12 is clear in osteoclast and chondroblast formation,<sup>37</sup> thus various studies investigated the polymorphisms in ADAM12 in different ethnicities.<sup>38–40</sup> SNPs rs1044122, rs1278279 and rs3740199 did not reveal any statistically significant association between the investigated polymorphism and the pathogenesis of KOA. The meta-analysis by Lv et al. confirmed rs1871054 in ADAM12 gene is linked to increased KOA in Chinese population.<sup>39</sup> Due to being upregulated in cartilage in KOA patients, its involvement in osteoclast differentiation and being a zinc dependent protein, GWAS investigations are required to study other polymorphisms in ADAM12 along with further confirmatory studies for rs1871054 using a larger sample size to investigate their contribution to KOA.

Growth differentiation factor 5 (GDF5) is a gene coding protein, involved in joint formation and bone morphology. GWAS revealed the association of the GDF5 rs143383 SNP with KOA in north American Caucasians,<sup>41</sup> similar results were observed in European Caucasians.<sup>42</sup> The variation is in the 5'UTR promoter region suggesting a stronger functional impact, Reynard et al. further confirmed this theory by identifying a methylation site as a result of the SNP rs143383 causing an allelic expression imbalance. The up regulated activity of the GDF5 gene mediated the increased susceptibility to OA.<sup>43</sup> Further investigation revealed an epigenetic difference between the GDF5 locus in the knee cartilage and the hip cartilage and results are more significant in the KOA.<sup>43</sup>

#### 4.3. Genes targeted for gene therapy

The bone morphogenetic protein (BMP) was investigated as a candidate gene for treatment and management of OA due to its role in cartilage development and calcium regulation and bone homeostasis<sup>44</sup> as well as being a subgroup in the TGF $\beta$  superfamily frequently associated with musculoskeletal conditions.<sup>29,45</sup> Although a study by Valdes et al. concluded that variations in BMP2 are linked to a reduction in KOA in females,<sup>46</sup> the decrease in BMP5 and BMP7 levels in the synovial fluid were often is a strong

contributor to the pathogenesis of OA.<sup>45,47</sup> The use of BMP7 in phase I of the clinical trials showed that the introduction of BMP7 into the site of injury has shown up to 70% after 24 weeks success rate in increasing cartilage thickness and decreasing general knee pain with the absence of any ectopic bone growth and toxic effect,<sup>48</sup> suggesting an increase in proteoglycans and collagen production. Blocking proteoglycans down regulation by inflammatory cytokines in the OA environment was indeed confirmed by Huck et al. using recombinant BMP.<sup>49</sup>

#### 4.4. Genes which need further investigation to understand their association with KOA

COL1A1 and COL1A2 are structural genes involved in the formation of the ECM.<sup>45,50</sup> An SNP rs16868943 in COL1A2 gene is associated with increased pain sensitivity to heat, pressure and punctate in KOA patients. Results also indicate that KOA patients carrying the same genotype, have lower tolerance to puncture in knees and arms, yet the results were inconclusive as they don't show statistical significance. Patients with such decreased pain intolerance should be advised to undertake regenerative medicine treatments in forms of intraarticular injections to avoid procedure-related painful adverse events.<sup>50</sup>

Mitochondrial DNA variation is suggested to be involved in the apoptosis of the cells in KOA. Mitochondrial dysfunction is suggested to be the cause of many other age-related degenerative disorders such as Alzheimer. Investigations by Fernández-Moreno et al., revealed the presence of a J haploidy in OA patients.<sup>51,52</sup> This haploidy is associated with increased risk of OA due to an increase in ROS and oxidative stress. The J haploidy slows the inflammatory cellular self healing process, and induces cellular apoptosis through wnt pathway. Patient with this haplotype might not benefit from regenerative medicine treatments as these methods aim to induce cellular healing.

## 5. Conclusion

To summarize all the material hereby presented in this literature review, it is possible to state that with all the preexisting observations made by researchers, multiple factors contribute to the progression of KOA, and no solid correlation between one single factor to KOA. Integrative therapies can focus on improving active transport by strengthening proper membrane barrier function and proper membrane bioenergetics so that active transport can be maintained, by supporting proper vascular endothelial function, and by enhancing cellular uptake through stimulation of membrane receptor sites and mitochondrial and nuclear signalling systems. While there is refined evidence reinforcing the importance of vitamin supplementation in the prevention of OA and enhance general bone health, research showed a counteracting effect of some minerals and vitamins on KOA, where other vitamins had no protective properties against KOA. In depth studies show the contributions of vitamin D insufficiency to the development of KOA. It is quite evident that estrogen and testosterone, the two main steroidogenic compounds in the human body, are both equally important in the physical development and maintenance of a healthy musculoskeletal system, however, it is now more apparent that estrogen is much more than just a primary female sex hormone involved in sexual development. In this review attention has been given to genetic contributions to KOA, especially because of the reported direct interactions with important bone genes, such as those responsible for osteoblasts, osteocytes and osteoclasts, cells which dictate bone formation or resorption. The identification of the genes contributing to KOA, raises the potential of utilizing the findings in designing gene therapy or identify the best treatment

strategy for treating patients with KOA.

In addition, regenerative medicine have been evaluated in KOA treatment and promising results were obtained, yet further optimization of the outcomes is required. To increase the success rates of regenerative medicine treatments on KOA, and due to their impact on the progression of the disease, its suggested to analyze the metabolic and nutritional profiles of KOA patients prior to treatment. Further research is required to confirm the contributions of each vitamin to supporting regenerative medicine. One suitable approach suggested by the evidence would be to modify the concentrations of hormones in a supplemental manner via methods such as hormone replacement therapy, providing the possibility to effectively modulate and prevent KOA.

### Conflicts of interest

The authors have none to declare.

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### Authors contributions

The authors' responsibilities were as follows KH search design, KH, JL, JM search. Extraction and content writing KH, JM, JL, AM, GS. Editing KH, JM. Formatting and referencing KH. KH had primary responsibility for final draft and submission, all authors read and approved the final draft manuscript, WDM draft feedback.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcot.2018.10.004>.

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