Vitamin D in NF1

Subjects: Oncology

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Vitamin D is a fat-soluble steroid hormone playing a pivotal role in calcium and phosphate homeostasis as well as in bone health. Several investigations indicated that vitamin D action extends far beyond bone health and calcium metabolism, showing broad effects on a variety of critical illnesses, including cancer, infections, cardiovascular and autoimmune diseases.

Epidemiological studies indicated that low circulating vitamin D levels inversely correlate with cutaneous manifestations and bone abnormalities, clinical hallmarks of neurofibromatosis type 1 (NF1).

NF1 is an autosomal dominant tumour predisposition syndrome causing significant pain and morbidity, for which limited treatment options are available.

Keywords: vitamin D ; vitamin D supplementation ; neurofibromatosis type 1 ; neurofibromin ; therapeutics

1. Vitamin D: Production, Dietary Intake and Supplementation

Endogenous production of vitamin D — occurring in the skin and triggered by sun exposure — is the most recognised natural source of this metabolite, accounting for 80–90% of vitamin D replenishment, thus labelling it as the ‘sunshine’ vitamin [1][2][3][4][5].

Vitamin D can also be exogenously obtained through dietary intake, which generally contributes only to 10% of the overall vitamin D levels [6][7]. Foods containing adequate levels of this micr[8][9][10][11][12]onutrient include cod liver oil and fatty fishes, such as swordfish, sardines, mackerel, salmon and tuna. Beef liver, egg yolks, yogurt and cheese represent additional natural sources of vitamin D, although contain it only in modest amounts [3][5][13].

Vitamin D supplementation represents the third and final source of vitamin D, available as over-the-counter products, for example, pills or oily drop preparations [14][15][16]. The use of oral supplements is particularly advised for people at risk of vitamin D deficiency, i.e., elderly, breastfed infants and individuals with dark skin, living in countries with low sunlight exposure or suffering from malabsorption syndromes [14][15][16].

2. Genetics of NF1: NF1 Gene and Neurofibromin

NF1 is inherited in an autosomal dominant mode and is caused by mutations in the NF1 gene, which is located on the long arm of chromosome 17, at q11.2. *NF1* gene spans over 350 kb of genomic DNA and comprises 59 constitutive exons and 4 alternatively spliced exons (i.e., 9a, 10a-2, 23a and 48a) [17][18][19]. In addition, it encodes a cytosolic protein of 2818 amino acids, called neurofibromin, which
acts as a tumour suppressor molecule [20][21].

The extreme clinical variability of NF1 disease suggests that random events intervene in determining the phenotype. Evidence in support of this interpretation is provided by the occurrence of somatic “second hit” mutation or loss of heterozygosity at the NF1 locus, that may influence the severity of the disease [22].

Neurofibromin controls growth, survival, proliferation and differentiation of cells mainly via two intracellular pathways. In particular, neurofibromin negatively regulates Ras activity and positively control adenylyl cyclase (AC) functions. It is a GTPase-activating protein (GAP) and exerts its functions acting as a negative regulator of the p21ras (Ras) proto-oncogene, promoting its conversion from the active GTP-bound Ras to its inactive GDP-bound form [23][24][25][26].

3. Vitamin D-NF1 Correlation: Clinical Evidences

Several studies reported the strict association between vitamin D levels and cutaneous or bone manifestations in NF1 patients.

Lammert and colleagues enrolled 55 adults with NF1 and 58 healthy controls, both men and women from Germany with a mean age of ca. 40 years old [27]. Circulating 25(OH)D levels were evaluated in all the selected subjects in autumn and winter since vitamin D amount is known to be season-dependent [28]. Compared to healthy controls, the mean distribution of 25(OH)D concentrations was found much lower in NF1 individuals. In addition, an inverse correlation between the serum levels of vitamin D and the number of dermal neurofibromas was reported in NF1 subjects [27].

More recently, the role of the vitamin D receptors (VDRs) was also explored by measuring mRNA levels in 141 NF1 adult patients [29]. This study demonstrated that the number of dermal neurofibromas inversely correlated with both VDR mRNA and serum vitamin D levels in NF1 subjects, further corroborating the previous findings [30] and suggesting that a low vitamin D content may contribute to the onset/development of the disease.[29].

Tucker et al. enrolled 72 NF1 adults and 312 healthy individuals from Germany [31]. Then, serum vitamin D and parathyroid hormone (PTH) concentrations in both groups were measured in summer and winter. Most of the NF1 subjects showed 25(OH)D and PHT levels outside the standard reference range, respectively lower and higher than season-matched controls in both summer and winter. Additionally, NF1 subjects also showed low bone mineral density (BMD) consistently with osteopenia or osteoporosis. These findings were sex-dependent since males showed reduced BMD more likely than females. Pathological fractures were also reported only in NF1 individuals [31].

In Utah (USA), Stevenson and co-workers recruited 109 children with NF1 and 218 healthy subjects, with a mean age of 10 years [32]. Similarly to previous studies [33], almost all NF1 individuals showed significantly reduced 25(OH)D levels than healthy individuals [32]. However, unlike previous observations [33], this study did not reveal a significant correlation between the reduction of vitamin D levels and the increase in the number of neurofibromas or optical gliomas in children with NF1 [32]. However, if we take due account of the age-related nature of neurofibroma formation, this relationship is more diffcult to assess in paediatric subjects. In addition, differences in vitamin D levels observed in paediatric and adult NF1 population could be a consequence of geographical location or feeding habits [32].

Besides the vitamin D status, Hockett and colleagues also evaluated the muscle function (in terms of power, force and height of their jump) in children with NF1 with respect to their unaffected siblings coming from Germany [34]. Thus, serum 25(OH)D and PTH levels weremeasured. Differing from Stevenson et al. [32], this study revealed no significant variation in vitamin D status between NF1 children and healthy controls, observing in all cases low mean 25(OH)D concentrations. Conversely, the mean PTH amount was significantly higher in children with NF1 compared to their unaffected siblings. In terms of muscular force, NF1 children showed impaired jumping power and force than healthy individuals [34].

In Canada, 18 children with NF1 were compared to their unaffected siblings in bone mineral content at the lumbar spine and proximal femur [35]. Subjects were selected between 6 and 20 years of age without focal bony lesions. Vitamin D and PTH levels were not significantly different between cases and controls, while NF1 subjects showed a sensibly reduced BMD compared to their healthy siblings. Additionally, affected children showed significantly lower bone strength, according to Hockett's group results [420], corresponding to a higher lifetime fracture frequency [35]

To obtain more insights on vitamin D levels and metabolism in NF1 patients, Schnabel and co-workers evaluated 25(OH)D amounts in German children and adults with NF1 in winter and summer and compared the results with those obtained for healthy individuals [36]. This study generally confirmed low levels of vitamin D in NF1 adults compared to healthy subjects. In addition, vitamin D levels in NF1 patients were higher in summer than in winter, with a better trend found in affected individuals than in the control group, but without the levels found in NF1 patients reaching those in healthy adults, showing that simple sun exposure seems unlikely to account for the observed differences [36]. On the other hand, in the case of the paediatric population, there were no significant differences in the amounts of 25(OH)D between affected and healthy children in both seasons, with similar improvements from winter to summer for both the analysed groups [36], according to the results obtained by Hockett et al. [32], in German patients, but in contrast with those reported by Stevenson and colleagues [37] and Armstrong et al. [35], who analysed respectively American and Canadian patients. The observed inconsistencies may reflect a different dietary intake of this nutrient since food fortification of vitamin D represents a very common approach in North America, but not in Germany.

In a recent investigation, Filopanti and colleagues proposed the trabecular bone score (TBS) as a tool for the measurement of bone microarchitecture and fracture risk in people with NF1 [38]. The authors determined vitamin D levels, vertebral and femoral BMD and TBS in 74 Italians with NF1 (26 males and 48 females) using a cohort of 61 voluntary

healthy subjects (16 males and 45 females) as control group [38]. TBS was found lower in NF1 individuals without differences between sexes. As expected, 25(OH)D levels and BMD in hip and spine were also lower in NF1 subjects compared to healthy controls [38]. In the NF1 group, there was an evident association between serum vitamin D concentrations and the number of dermal neurofibromas, confirming previously reported data [30]. On the contrary, no correlations between TBS and 25(OH)D or the number of cutaneous neurofibromas were found [39].

In another study, along with the vitamin D levels, also VDR FokI and BsmI gene polymorphisms were examined [40]. Both FokI and BsmI polymorphisms can generate a decreased VDR expression [41][42][43][44], which in turn may reduce vitamin D effects, even in the presence of normal vitamin D levels. In 45 adults with NF1 (18–72 aged) from Southern Brazil, vitamin D amounts were measured and compared with those of 45 healthy controls matched by sex, skin type and age [45]. The differences in vitamin D levels between NF1 patients and healthy subjects were found not statistically significant, even if NF1 individuals showed reduced vitamin D levels. In particular, two patients with the lowest vitamin D levels also showed the largest number of cutaneous neurofibromas, corroborating the previous outcomes of Lammert et al. [30].

4. Treatment of NF1: The Use of Vitamin D Alone or in Combination Therapy

The unambiguous correlation between vitamin D levels and NF1 clinical features stimulated the use of vitamin D or its analogues as therapeutic agents as well as their combination with well-established Ras-pathway inhibitors for NF1 treatment.

Nakayama and colleagues isolated primary fibroblasts from cutaneous neurofibromas of NF1 patients and demonstrated a remarkable cell growth reduction after treatment with vitamin D3 or its analogues, i.e., tacalcitol (1,24-dihydroxyvitamin D3) or 22-oxacalcitriol (22-oxa-1,25-dihydroxyvitamin D3), also known as OCT [46].

Subsequently, fibroblasts, mast cells and Schwann cells were isolated from neurofibromas and their in vitro cellular growth was evaluated after vitamin D3 treatment and/or narrowband UVB (NB-UVB) irradiation [430]. The use of light irradiation (308 nm) sensibly reduced the proliferation of all the cell types, whereas the exposure to vitamin D3 or its synthetic analogue tacalcitol was effective only on the proliferation of fibroblasts and mast cells. A combination of calcitriol or tacalcitol with light irradiation provided additive effects on the cultured cells [47]. These results suggested that the response to vitamin D3 is cell specific and fibroblasts are the most sensitive cells [47].

On Schwann cells and fibroblasts isolated from neurofibromas, the same research group also examined the effect of rapamycin (or sirolimus), i.e., an mTOR inhibitor and lovastatin — a Ras-MEK pathway inhibitor — alone or in combination [48]. Schwann cells' growth was reduced either by the use of rapamycin or lovastatin in a dose-dependent manner, whereas their combination resulted in additive inhibitory effect. Similar outcomes were also observed for fibroblasts although with effect slightly lower than those reported in Schwann cells. A combination of vitamin D3 with rapamycin and/or lovastatin was also explored: the use of calcitriol slightly strengthened the efficacy of either drug in Schwann cells, while in fibroblasts additive effects were found [48].

In a different study, neurofibroma tissue was transplanted subcutaneously into nude mice skin and OCT was administered. With respect to the untreated mice, the growth and the density of neurofibroma tissue was found to be sensibly reduced by daily, local OCT injection [49]. Subsequently, the topical application of OCT to nude mouse skin for six months proved to be effective in reducing the pigmentation of café au lait spots [50].

Stimulated by these intriguing in vitro and in vivo findings, the same research group also investigated the effects of NB-UVB irradiation on the serum vitamin D levels in NF1 patients [434]. Once weekly or biweekly, NB-UVB irradiation proved to markedly increase the serum 25(OH)D levels, with detectable di erences after 18 months between treated and untreated groups [51]. Time-course analyses of the serum 25(OH)D levels in the treated NF1 patients revealed that the overall concentrations became significantly higher after 6 months of irradiation, then reaching a plateau since the prolonged treatment did not provide any additional beneficial effect [51].

The same research group also evaluated the efficacy of intense pulsed-radiofrequency (IPL–RF) combined with the topical application of OCT ointment (Maxacalcitol, OXAROL®, Chugai Pharmaceutical, Tokyo) for the treatment of NF1 pigmented lesions [52]. Indeed, IPL–RF was proved to be absorbed by melanin pigments producing their direct destruction [53]. First, the authors reported a single case of a 27-year-old woman successfully treated over 4 months with this combination therapy: indeed, an increased lightness of her skin was detected [54]. Thus, the authors extended the investigation to 8 NF1 patients (2 males and 6 females) aged 3–38 years (with a mean age of 20 years), which were treated for almost 2 years in different body sites such as face, neck, trunk and legs. IPL–RF/OCT combination improved

the appearance and reduced the number of small pigmented freckling more than CALMs, with a moderate to good response in six of the eight treated patients [435]. Furthermore, no topical or local anaesthesia was used during IPL–RF exposure and no re-pigmentation was observed for the successive 6 months after treatment [52].

Since supplementation with calcium and vitamin D3 demonstrated beneficial effects on BMD, this combination was also evaluated in NF1 patients with general bone abnormalities [55][56]. Brunetti-Pierri et al. investigated bone status in 73 NF1 subjects, 26 males and 47 females, mainly children and adolescents (mean age of ca. 16 years) [57]. In a subgroup of 16 subjects with marked osteoporosis and osteopenia, they found a statistically significant and generalised reduction in bone mass compared to normal controls. Additionally, in this subgroup, 8 individuals also showed slightly higher serum PTH concentrations and 10 patients had a serious vitamin D insufficiency. These subjects were specifically selected to measure bone turnover and bone density before and after vitamin D3 and calcium treatment [57]. After 4 months of supplemental therapy, PTH was increased to normal levels in 6/8 subjects, but lumbar spine BMD did not significantly change over two years [57]. In contrast, subsequent investigations proved the combination of vitamin D3 and calcium beneficial for BMD improvement.

Seitz and colleagues examined 14 adults (5 men and 9 women) affected by NF1, aged in the 19–66 years range, with an average age of ca. 44 years [58]. The control group included 15 males and 27 females with a mean age of 47 years. For NF1 subjects, histologic analysis of iliac crest biopsies revealed an increased osteoid volume associated with a higher number of osteoblasts and osteoclasts compared to biopsies from healthy individuals. Additionally, NF1 patients showed significantly lower 25(OH)D serum levels and decreased BMD with respect to healthy controls, accompanied by high PTH levels. Hence, a combination of vitamin D3 and calcium was administered for one year in a subgroup of 4 patients with remarkable reduced BMD [58]. After this treatment, both vitamin D and PTH serum levels were normalised and a significant improvement in BMD in the spine but not in the hip was observed [58].

Similar outcomes were also achieved by Schnabel and colleagues, who focused on the effects on the hip and lumbar spine of adult NF1 patients with vitamin D3 deficiency [59]. The serum levels of 25(OH)D and BMD were determined in 35 adult subjects with NF1 (12 men and 23 women, with age ranging from 32 to 63 years). 19 patients received vitamin D3 supplementation for 2 years, 6 patients for one year and 10 patients no received supplementation [59]. Compared to untreated individuals, treated subjects showed a significantly improved BMD especially at the level of the hip [59].

The evaluation of bone mineral metabolism parameters in NF1 patients, before and after calcium and vitamin D3 supplementation, was also performed by Petramala and colleagues [60]. The authors evaluated 70 adult NF1 patients (37 men and 33 women, mean age ca. 40 years) and 40 normal subjects (22 men and 18 women with a mean age of ca. 44 years). Individuals affected by renal failure, cardiovascular or thyroid dysfunctions were excluded from this study [339]. A total of 35% of NF1 patients showed bone alterations featured by reduced BMD of the lumbar spine and femoral neck associated with an increased prevalence of osteopenia or osteoporosis. Moreover, NF1 individuals exhibited severe hypovitaminosis D and high PTH levels than the control group [60]. For the first time, reduced magnesium levels were reported in NF1 patients: magnesium is important for bone health since its reduction can promote the development of reduced bone mass [60]. After one year of supplementation of calcium and vitamin D3, a significant increase in 25(OH)D and magnesium levels, a sensible reduction pf PTH levels and general improvements in the bone mass were observed [60].

In a subsequent study, 6 patients with NF1-related osteoporosis were enrolled to evaluate the efficacy of vitamin D3 treatment in combination with alendronate [61]. Alendronate is a bisphosphonate medication able to target osteoclasts and inhibit farnesyl diphosphate synthase, interfering with the farnesylation of small GTPases including Ras, Rac and Rho [62] [63]. This process leads to osteoclast apoptosis, an increase in BMD and a reduction in fracture risk [446,447].

Alendronate is used for the prevention and treatment of osteoporosis [448]. In this study, for almost two years, a weekly dose of alendronate and a daily vitamin D3 supplementation was administrated to 5 men and 1 woman, aged 28–76 years [64]. After the treatment, BMD was increased in 5 out of 6 patients, but this increase was not statistically significant. A new stress fracture of the tibia was also documented in a patient under therapy. Unfortunately, this investigation did not provide unambiguous results on the effects of vitamin D3/ alendronate combination on NF1-related osteoporosis patients because of the low number of enrolled individuals [64].

Regarding the clinical trials, the U.S. National Institutes of Health reports only one vitamin D supplementation study in NF1 patients (searching for “neurofibromatosis” and “vitamin D”). The aim of this investigation (NCT01968590) was to evaluate the treatment of adult NF1 patients (25–40 years old), who show insufficient serum 25(OH)D levels, with two different doses of vitamin D supplementation over 2 years and to verify if improvement in the BMD loss can be achieved over this time. To the best of our knowledge, the results of this study are not yet available.

5. Conclusions and Perspectives

The metabolism of vitamin D — regulating a broad spectrum of physiological processes — proved to play a key role in the pathogenesis of NF1 disease. Thus, personalised nutrition including a tailored vitamin D supplementation may represent a very useful approach in the maintenance of wellbeing of NF1 patients and their management.

In NF1 individuals, vitamin D deficiency can worsen bone metabolism, promoting reduced bone mass and general bone abnormalities. Thus, the restoration of healthy vitamin D status can be an effective therapeutic intervention in NF1 patients.

Individuals with a huge number of dermal neurofibromas can be more prone to cover their skin for aesthetic embarrassment or discomfort and decrease their outdoor activities, especially in the presence of associated NF1 comorbidities (e.g., scoliosis, pseudoarthrosis).
Clothing selection and lifestyle habits of NF1 patients can affect the sunlight irradiation usually received, or conversely, the increased pigmentation of their skin negatively influences the efficiency to produce an adequate amount of vitamin D, even in the presence of an adequate light exposure.

To better understand the vitamin D levels in NF1 patients, studies on vitamin D intake, absorption, synthesis, transport, or catabolism in affected individuals could be very useful.

It would also be interesting to explore a large variety of dietary supplementation based on the combination of vitamin D with other healthful agents — for example, polyphenols, known to exert neuroprotective functions [65][66][67] — with the aim to test possible synergistic effects.

Moreover, vitamin D has been also incorporated — using calcitriol or some of its analogues as active components — in different drug delivery systems for food fortification or therapeutic applications [68][69][70]. To the best of our knowledge, these micro/nano-formulations have not been tested in NF1 patients; so this strategy can be attempted in the next future to improve NF1 patient management and their life quality.

As NF1 remains a multisystem disease with life-threatening complications, a multidisciplinary approach with close collaboration among clinicians and researchers will be needed for the diagnosis and management of this condition.

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