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Review Article

Does the environment influence multiple sclerosis pathogenesis via UVB light and/or induction of vitamin D?

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ABSTRACT

Multiple sclerosis (MS) is a disease of presumed auto-immune origin. Long-standing observations such as the correlation between MS incidence and geographical latitude or the levels of Vitamin D (Vit D) in the serum have implicated the environmental factors UVB radiation and diet in the etiology of the disease. Clinical trials have been conducted and are currently underway to elucidate whether a Vit D enriched diet or treatment with UVB can influence MS incidence, −severity, and −progression, as well as the ideal time point for treatment. This review summarizes the current scientific knowledge to the environmental factors UVB-light and Vit D concerning the clinical aspects of MS in epidemiological studies and clinical trials.

1. Introduction

The etiology of multiple sclerosis (MS), an inflammatory and neurodegenerative demyelinating disease of the central nervous system (CNS), which is most likely of autoimmune origin (Sospedra and Martin, 2016), is still not completely understood. It is currently considered as a very heterogeneous (spectrum) disorder where multiple factors, both genetic and environmental, contribute to disease susceptibility and individual course (Ascherio and Munger, 2016). MS is characterized by a striking geographic variation of prevalence and incidence with a positive latitude gradient - the further away from the equator, the higher the disease incidence (Simpson et al., 2011; Taylor et al., 2010; Risco et al., 2011). In the 1960ies, this lead many to believe in a link between low levels of sun exposure and an increased risk of developing MS (Acheson and Bachrach, 1960). Since UVB irradiation of the skin is necessary to produce active Vitamin D (Vit D) from its precursor (**see** Box 1), why Vit D is also called the “sunshine” vitamin (Wacker and Holick, 2013; Nair and Maseeh, 2012), Vit D has subsequently been postulated by many investigators (Goldberg, 1974) as a major intermediary link between sunlight exposure and the risk of developing MS. Accordingly, hypovitaminosis D has indeed been recognized as one of the environmental factors most consistently associated with MS susceptibility (Pierrot-Deseilligny and Souberbielle, 2017; Hanwell and Banwell, 2011). In parallel, several studies indicate, that UVB light exerts beneficial effects in reducing the risk of MS, but those might be effects independent from Vit D synthesis (reviewed in (Hart and Gorman, 2013, DeLuca and Plum, 2017)) and, thus, the benefits of supplementation with Vit D have not been definite (see clinical trials). Vit D supplementation in patients with existing MS has not yet been shown beyond doubt to alter disease activity, −course, or -susceptibility in large-scale clinical studies.

The present review provides a brief outline on the effects of UVB light exposure on MS risk and progression that are likely to be dependent of the synthesis of active Vit D and on those that are independent from Vit D. We will furthermore summarize the current state of clinical trials investigating the effects of Vit D supplementation and UVB treatment in MS.

1.1. Vitamin D (deficiency) and MS susceptibility

Extensive epidemiological research supports the causality of the relationship between low Vit D levels and the onset and development of MS. Correlative links between an insufficiency in Vit D and/or sunlight exposure during the first part of life and an increased risk of developing MS later in life have been reported: a month-of-birth effect with higher than expected risk for those born at the end of spring and conversely lower risk for those born at the end of autumn has been used to suggest that maternal exposure to environmental UVB levels during pregnancy is involved in risk determination (Balbuena et al., 2016; Rodriguez Cruz et al., 2016; Dobson et al., 2013). This is supported by a recent Finnish study which found an association between maternal Vit D deficiency (serum Vit D levels < 30 nmol/l) during early pregnancy and a 90% enhanced MS risk of the offspring later in life (Munger et al., 2016). Furthermore, higher Vit D serum levels in newborns have been reported

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to provide protective effects for the MS risk later in life, whereas low neonatal Vit D levels are associated with an increased risk of MS (Nielsen et al., 2017). These findings suggest that lower Vit D levels during early life (prenatal and neonatal phase) could mark a susceptibility period independent from the phase childhood, as children who move early in life adapt to the MS risk of their country of upbringing (Handel et al., 2010; McLeod et al., 2011; Barnett et al., 2016). However, higher Vit D levels in utero and neonatal levels do not necessarily reflect a direct protective effect, but could also reflect a possible correlation between neonatal Vit D levels and Vit D serum levels later in life, linked by similar behavior between mother and child, e.g. time spent outside, as well as genetic factors. Nonetheless, all these associations implicate that correction of maternal Vit D deficiency during pregnancy (e.g., by appropriate diet or low-dose UVB radiation) would be a potential option to reduce MS risk in the offspring. Studies indicate that Vit D supplementation with moderate oral doses (up to 4.000 IU/day) is safe in pregnant women (Wagner et al., 2017). However, Vit D produced in the skin usually lasts twice as long in the blood compared to ingested Vit D (Haddad et al., 1993). Generally, it is considered that in an adult, who is exposed to just one minimal erythemal dose of UVB light, the amount of vitamin D produced is equivalent to ingesting between 10.000 and 25.000 IU (Holick et al., 2007; Nair and Maseeh, 2012).

Prospective epidemiological studies have further established Vit D as a key environmental factor associated with MS risk: high serum Vit D levels among healthy young adults correlated with a decreased risk for developing MS later in life. In a cohort of US soldiers those with a Vit D status of ≥100 nmol/l had a 62% decreased risk of later developing MS (Munger et al., 2006). Similarly, a study conducted in a cohort of Swedish pregnant women found that Vit D levels ≥75 nmol/l were associated with a 61% lower MS risk (Salzer et al., 2012). These findings were recently confirmed by the largest longitudinal investigation to date assessing in the Finnish Maternity Cohort whether Vit D in healthy individuals predicts risk of MS later in life: women with Vit D levels ≥50 nmol/l had a 39% reduced risk of developing MS (Munger et al., 2017).

1.2. Vitamin D and MS activity or -progression

Several studies have demonstrated that Vit D exerts relevant immunomodulatory functions (Dankers et al., 2016; Pierrot-Deseilligny and Souberbielle, 2017; Hart et al., 2011). Therefore, Vit D seems not only to be an environmental factor associated with risk of developing MS, meaning that exposure to insufficient Vit D levels occurs before the onset of MS, but also appears to modulate disease activity and progression during established MS. Pathogenetically this are two categorical different scenarios: on the one hand immune tolerance networks associated with susceptibility for MS and on the other hand immune regulatory factors modulating existing autoimmunity. Low serum Vit D levels have repeatedly been associated with an increased relapse rate and progression of MS, whereas a sufficient Vit D status predicted a slower rate of progression and reduced MS activity (Ascherio et al., 2014; Mowry et al., 2012; Runia et al., 2012; Fitzgerald et al., 2015; Laursen et al., 2016). Moreover, Vit D deficiency could be associated with more rapid acquisition of neurological disabilities in MS: low Vit D levels correlated with a higher degree of disability in fully ambulatory patients with RRMS (Thouvenot et al., 2015).

An association study testing serum Vit D levels early in the course of MS and putting them into relation with clinical and MRI outcomes after 5 years showed that high serum levels in the initial phase of the disease were associated with lower degree of MS activity and progression as well as MRI lesion load and brain atrophy over 5 years of follow-up (Ascherio et al., 2014). These observations promote the concept of Vit D exerting beneficial effects on inflammatory components in MS pathology, as well as in the initial disease phase. A study by Martinelli (Martinelli et al., 2014) reporting that low Vit D levels in patients with a clinically isolated syndrome (CIS) are associated with increased risk of developing clinically definite MS further supports a protective effect of Vit D in the early phase of MS. Experimental studies have demonstrated that Vit D exerts broad immunomodulatory properties and has various biological effects on the immune system. Since summarizing the mechanisms of Vit D mediated immune modulation would go beyond the scope of this review, we here refer to some extensive reviews on this topic (e.g. (Hart et al., 2011, Dankers et al., 2016)).

1.3. Genetically induced hypovitaminosis D as a potential risk factor for MS

Low Vit D levels in MS patients might not only be determined by inadequate sun exposure due to sociocultural and lifestyle factors, as well as latitude and seasonal variation or insufficient dietary uptake of Vit D, but also by genetic factors. Genome-wide association studies (GWAS) have identified genetic abnormalities of genes involved in Vit D metabolism, to be specific CYP27B1, encoding 1α-hydroxylase, the enzyme performing the conversion of Vit D into its active metabolite, and CYP24A1, encoding 24-hydroxlase, the enzyme converting the active Vit D into its inactive metabolite calcitroic acid, to be associated with an increased risk of MS (International Multiple Sclerosis Genetics et al., 2011). Additional studies, aiming to link genetic hypovitaminosis D to MS risk, have identified single nucleotide polymorphisms (SNPs) that are associated with low serum Vit D levels. A Mendelian randomization study using data from the SUNLIGHT study (Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits), the largest GWAS to date for Vit D, has identified four SNPs (rs10741657, rs12785878, rs2282679, rs6013897) that are associated with lower serum Vit D levels and an increased susceptibility to MS (Mokry et al., 2015). A relationship between SNPs associated with genetically lower serum Vit D levels and MS risk was also reported in a study by Rhead et al. (Rhead et al., 2016) using Mendelian randomization in 2 independent populations, an American and a Swedish population, from areas with different environmental levels of UVB radiation. These findings indicate that Vit D insufficiency contributes to MS susceptibility both as an environmental and as a genetic risk factor. Interestingly, genetic variation in Vit D binding protein (SNP rs4588 or its proxy rs2282679) has further been found to result in a smaller serum Vit D increase after vitamin D3 supplementation as compared to individuals without the SNP (Nimitphong et al., 2013), demonstrating some pharmacokinetic implications in potential supplementation regimens. In this line, the responsiveness to Vit D supplementation seems to also be impaired in MS patients: a recent study investigating whether Vit D supplementation results in similar increase in serum Vit D levels in healthy controls and MS patients showed that people with MS had a lower increase in Vit D levels following oral vitamin D supplementation as compared to healthy controls (Bhargava et al., 2016). These findings might support assumptions that are the basis of the so-called “Coimbra-protocol” claiming that MS patients (and other autoimmune diseases) need higher doses of Vit D to reach adequate levels due to a genetically caused hypovitaminosis D (Finamor et al., 2013). As there are no peer-reviewed study results available for the effects of the “Coimbra protocol” (doses of Vit D up to 400.000 IU!), it is currently unclear if ultra-high doses of Vit D really have the desired disease-modifying effect, nor if the higher risk of side effects from ultra-high doses is worth the benefit. To date there is no study available to link the hypothesis of a Vit D “resistance” in MS patients with a genetic or pharmacological explanation: the study by Bhargava et al. did not investigate, whether MS patients included in the study are carriers of SNPs associated with low serum Vit D levels. Therefore, further investigations in clinical vitamin supplementation regiments and controlled trials are needed; assessment of relevant SNPs might indeed be useful to adjust treatment in MS patients with an insufficient response to Vit D supplementation (pharmacogenomics of Vit D supplementation).

Of note, an exciting study in a cohort of disease-discordant monozygotic twins addressing the role of childhood sun exposure on MS risk

indeed, observed a strong inverse association between the appearance of MS and the relative frequency of sun exposure independent of genetic susceptibility (Islam et al., 2007). The importance of exposure to sunlight among individuals with identical genetic susceptibility is illustrated by this study, and thereby emphasizes that Vit D insufficiency can be both an environmental and/or a genetic risk factor for MS.

1.4. Clinical assessment and -trials of vitamin D in MS

Albeit the association between Vit D and MS susceptibility, as well as a link between Vit D deficiency and disease progression, are epidemiologically well established, the use of Vit D supplementation for therapeutic intervention is still a matter of controversy. Small interventional trials investigating the therapeutic potential of Vit D for treating established MS have revealed inconsistent results concerning clinical outcomes and outcomes on surrogate parameters such as MRI (e.g.(Soilu-Hanninen et al., 2012) study duration: 1 year, enrolled subjects: 66; (Stein et al., 2011) study duration: 6 months, enrolled subjects: 23; (Kampman et al., 2012) study duration: 96 weeks, enrolled subjects: 68; (Laursen et al., 2016) study duration: 2 years, enrolled subjects: 170; (Mowry et al., 2012) study duration: 5 years; enrolled subjects: 469; just to name a few). However, none of these studies was powered sufficiently to measure treatment effects and the study duration of some trials was rather short. Therefore, high quality controlled trials of Vit D supplementation in MS are still needed to establish solid evidence for a therapeutic effect of Vit D administration. Several larger, randomized clinical trials of Vit D supplementation for both **onset and activity** in MS are currently underway or have recently been completed. An exhaustive review of all conducted trials (for an overview see ClinicalTrials.gov) is beyond the scope of this paper, but the most prominent studies are discussed here (see also Table 1).

- **VitD4MS** (“Safety trial of high dose oral vitamin D3 with calcium in multiple sclerosis”; NCT00644904; phase I/II randomized open-label trial) was the first trial examining the safety/tolerability of high oral Vit D doses (up to a maximum of 40.000 IU daily) in MS patients over 52 weeks. 25 patients assigned to the treatment group received escalating doses from 4.000 IU up to 40.000 IU Vit D per day for the first 28 weeks followed by a down-titration to 4.000 IU per day in the second half of the study and a complete discontinuation at the end of the trial period. A control group consisting of 24 patients was restricted to a maximum of 4.000 IU per day. High-dose Vit D administration was well tolerated in MS patients: no significant adverse events occurred in the patients on high-dose supplementation and serum calcium levels were not significantly increased compared to patients in the control group (Burton et al., 2010). These findings indicated that high dose Vit D supplementation, with a mean of roughly 14.000 IU per day, is safe in MS. However, it has to be mentioned that the study was rather short and that even though no adverse events emerged, the long-term effects of high-dose Vit D levels are not yet known, especially since Vit D is a steroid hormone. Patients treated with high-dose Vit D further appeared to have fewer relapse events and T-cell reactivity and -proliferation decreased compared to controls (Kimball et al., 2011a). However, the study was neither blinded nor powered enough to properly address clinical outcomes and study results have, therefore, to be considered as preliminary evidence for beneficial Vit D effects.
- **VIDAMS** (“Vitamin D to Ameliorate Multiple Sclerosis”; NCT01490502; randomized, double-blind clinical trial; USA) is evaluating the benefits of low-dose (600 IU daily) versus high-dose (5.000 IU daily) oral Vit D supplementation as add-on to glatiramer acetate (Copaxone) in 172 RRMS patients over a duration of 96 weeks. Results assessing the impact of Vit D on the number of relapses, new lesions on brain MRI and change in brain volume are expected in March 2019; interim analyses are not planned. Particularly interesting is the combination of Vit D and glatiramer acetate in this study, as Vit D as add-on therapy to glatiramer acetate has not been investigated before. Additionally, the low-dose (600 IU daily) Vit D supplementation in the control arm is the daily dose recommended by the US Food and Nutrition Board, which according to pharmacokinetic studies, however, does not result in substantial Vit D serum level raise (Bhargava et al., 2014).
- **EVIDIMS** (“Efficacy of Vitamin D Supplementation in Multiple Sclerosis”; NCT01440062; interventional, randomized, controlled and double-blind clinical phase II pilot study; Germany) assesses the efficacy of either high-dose (20.400 IU every other day) or low-dose (400 IU every other day) Vit D supplementation in eighty patients with RRMS or CIS, who are on a stable immunomodulatory treatment with interferon-β1b over a study period of 18 months by determining the cumulative number of new T2-weighted cranial MRI lesions during the trial. For the control arm a low-dose Vit D administration, which most probably has no immunomodulatory potential, was chosen due to ethical considerations and for the high-dose arm a daily administration of 10.200 IU was selected as it represents the maximum dosage for which sufficient safety data were available at the start of the study (Dorr et al., 2012). The EVIDIMS study has been completed; results, however, have not been reported so far.
- **SOLAR** (“Supplementation of VigantOL Oil versus placebo as add-on in patients with relapsing remitting multiple sclerosis receiving rebif treatment”; NCT01285401; randomized, double-blind, placebo-controlled) with 229 included patients was the largest completed trial so far, assessing whether Vit D (cholecalciferol) as add-on therapy has any benefit on the progression of MS in RRMS patients receiving subcutaneous interferon-β1a (Rebif). Patients in this 48-week study, whose serum Vit D level was below 150 nmol/l, received a daily Vit D dose of 7.000 IU in the first 4 weeks and thereafter, if the treatment was well tolerated, 14.000 IU for a further 44 weeks or a placebo over the full study-period in case they were randomized to the control group (Smolders et al., 2011). The primary endpoint, percentage of subjects with a disease-activity free (DAF) status at week 48, which was defined as the absence of relapses, no expanded disability status scale (EDSS) progression or new combined unique (CUA) lesions, was not met. At the end of the study, there was no significant difference in DAF status between the cholecalciferol supplemented group and the placebo group (37.2% versus 35.3%; $P = .912$). Although the primary endpoint measure was not improved by Vit D, a beneficial effect of the supplementation was suggested by imaging results: at week 48, a reduction of 32% in the number of new CUA lesions was observed for the cholecalciferol treated group as compared to the placebo group ($P = .005$). In the subgroup of patients aged 18–30 years, the percentage of MS patients being free from new T1 hypo-intense lesions was also higher for the Vit D supplemented group compared to the placebo treated group (85.7% versus 46.8%; $P = .006$). (Smolders, 2016; Hupperts, 2017; Muris et al., 2016).

The lack of significance for the primary endpoint should be interpreted in light of the relatively short study duration with 48 weeks and the still relatively small cohort size with 229 patients. Even though the SOLAR study is the largest completed trial of Vit D as add-on therapy in MS so far, its proper comparisons are randomized trials of new MS drugs, which often recruit over 900 patients (e.g. (Gold et al., 2012, Polman et al., 2006, Kappos et al., 2015); to demonstrate a significant effect. Furthermore, the beneficial effects of Vit D supplementation on MS progression might be more pronounced in early stages of the disease, where intense inflammatory activity is likely. In fact, there are currently 2 clinical trials (**PrevANZ and D-Lay-MS**) underway, investigating the effect of Vit D supplementation on patients with a first demyelinating event. The **CISAVID** trial (“Dose-related effects of vitamin D3 on immune responses in patients with clinically isolated

Table 1
Important clinical trials involving Vitamin D supplementation or phototherapy.

Trial name	Cohort location	Study type	Treatment & arms	Number of enrolled/ estimated subjects	MS type	Associated treatment	Study duration	Status/estimated end of study	References
VitD4MS NCT00644904	Canada	Interventional Phase I/II Randomized Open-label	– Treatment group (25 patients) Vit D_3 titrated up to a max. of 40.000 IU/day – Control group (24 patients) allowed to supplement with up to 4.000 IU/day	49	RRMS or SPMS	Interferon beta-1a (Avonex, Rebif) interferon beta-1b (Betaferon); glatiramer acetate (Copaxone)	52 weeks	Completed	Kimball et al. (2011a); Kimball et al. (2007); Kimball et al. (2011b)
VIDAMS NCT01490502	USA	Randomized Double-blind Controlled	– High dose group 5.000 IU Vit D_3/day – Low dose group 600 IU Vit D_3/day	172	RRMS	Glatiramer acetate (Copaxone)	96 weeks	March 2019	Bhargava et al. (2014)
EVIDIMS NCT01440062	Germany	Interventional Phase II pilot study Randomized Double-blind Controlled	– High dose group 20.400 IU Vit D_3/every other day – Low dose group 400 IU Vit D_3/every other day	80	RRMS or CIS	Interferon beta-1b	72 weeks	Study has been terminated	Dorr et al. (2012)
SOLAR NCT01285401	Europe	Phase II Randomized Double-blind Placebo-controlled	– Treatment group 7.000 IU Vit D_3/day for the 1st 4 weeks 14.000 IU Vit D_3/day for 44 weeks – Placebo group matching placebo daily for 48 weeks	229	RRMS	Interferon beta-1a (Rebif)	48 weeks	Completed	Smolders et al. (2011); Muris et al. (2016)
CISAVID NCT01728922	Ireland	Interventional Randomized Double-blind Placebo-controlled	– 5.000 IU Vit D_3/day – 10.000 IU Vit D_3/day – placebo	45 CIS patients 39 Healthy controls	CIS	No DMTs	24 weeks	Completed	O'Connell et al., 2013)
D-Lay-MS NCT01817166	France	Interventional Randomized Double-blind Placebo-controlled	– Treatment group 100.000 IU Vit D_3 every 14 days – Placebo group placebo treatment every 14 days	316	CIS	Not specified	Max. 2 years or until conversion to full MS	January 2022	ClinicalTrials
PrevANZ ACTRN 12612,001,160,820	Australia, New Zealand	Interventional Phase IIb Randomized Double-blind Placebo-controlled	– 1.000 IU Vit D_3/day – 5.000 IU Vit D_3/day – 10.000 IU Vit D_3/day – placebo capsule daily	240	CIS	Without any DMT other than glucocorticoids	48 weeks	End of 2019	ANZCTR
PhoCIS ACTRN 12614000185662	Australia	Interventional Randomized Open-label	– Treatment group narrow band (311 nm) UVB light therapy; 3 times per week for 8 weeks – Control group no UVB therapy all participants receive Vit D supplementation to achieve serum Vit D levels of ≥80 nmol/l	60	CIS	DMT-free	8 week treatment; 12 months of follow-up	Anticipated date of last Enrollment: end of 2016	Hart et al. (2017)
Phototherapy in persons with MS NCT02365259	USA	Interventional Randomized Triple-blind Placebo-controlled	– Treatment group UVB phototherapy 3 times per week for 8 weeks – Placebo group Shame phototherapy 3 times per week for 8 weeks	20	MS	Not specified	8 weeks	Unknown	ClinicalTrials

MS: multiple sclerosis; CIS: clinically isolated syndrome; DMT: disease-modifying therapies; RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; Vit D: Vitamin D.

syndrome"; NCT01728922; interventional, double-blind randomized placebo-controlled trial) which also aimed to examine the immunologic effects of Vit D supplementation at two doses (5.000 IU or 10.000 IU daily) in patients with CIS over a 24-week treatment period (O'Connell et al., 2013), has been completed, but no results of the study have been reported yet.

- **D-Lay-MS** ("Efficacy of Cholecalciferol (Vitamin D3) for Delaying the Diagnosis of MS After a Clinically Isolated Syndrome"; NCT01817166; interventional, randomized, double-blind versus placebo study; France) evaluates the efficacy and tolerance of Vit D treatment in an estimated 316 patients with CIS, who are at high risk for developing MS. Patients receive either 100.000 IU of Vit D or a placebo treatment mimicking Vit D application for a maximum of 2 years or until conversion to clinically definite MS has occurred. The study is currently recruiting participants and first results on the primary outcome measure, namely the conversation rate to clinically definite MS in the two treatment arms, are expected in January 2022. This study analyzes the effects of Vit D in a phase, when the disease course might be more susceptible to intervention and will therefore provide new insights on the efficacy of Vit D in deviation or slowing of MS development.
- A similar study, the **PrevANZ** ("Preventing the risk of Multiple Sclerosis using Vitamin D in patients with a first demyelinating event in Australia and New Zealand"; ACTRN12612001160820; phase IIb, randomized, double-blind, placebo-controlled trial) is currently recruiting an estimated 240 CIS patients in Australia and New Zealand. The trial tests the efficacy and safety of different Vit D doses (1.000 IU vs 5.000 IU vs 10.000 IU), that are administrated daily for 48 weeks, in comparison to a placebo control in preventing or delaying the conversion into clinically definite MS. The anticipated completion of data collection is end of 2019.

Collectively, there is evidence from clinical trials and epidemiological studies that insufficient Vit D levels might increase the risk for MS and adversely affect its progression. Vit D could therefore represent a simple target for MS prevention and therapeutic modification. However, the results from currently running trials have to be awaited, as further investigations are needed to identify optimal levels of Vit D supplementation as well as the optimal window of intervention (e.g. trials with CIS patients) and the optimal duration of treatment. It is still debatable whether there is a critical timing for Vit D supplementation, as insufficient Vit D levels have been identified as MS risk factor during various stages in life (childhood, adolescence, adulthood and/or even in utero). Additionally, for the use of Vit D as an add-on therapy for MS treatment it is crucial to evaluate, whether Vit D supplementation has beneficial effects only during the initial phase of the disease or also upon established MS.

As an additional caveat, most of the clinical trials on Vit D have been conducted in white populations from the United States and Europe. Therefore, further investigations are needed to evaluate whether study results might apply to different races, gender, or ethnicities, especially since the expected effect size of Vit D supplementation at best is moderate. Potential confounders such as genetic factors should be included in the study evaluations as well and longer follow-up durations would be favorable for more conclusive experimental evidence of the beneficial actions of Vit D. Additionally, to minimize the effects of any intervention in clinical Vit D trials it would be important to recruit study participants in months with low environmental levels of UVB radiation and to monitor the amount of time spent outdoors by the participants. That way the effects of the supplemented Vit D can be monitored more effectively without being confounded by the effects of sunlight exposure. As Vit D produced in the skin upon UVB exposure lasts twice as long in the blood compared to supplemented or ingested Vit D (Haddad et al., 1993), differences in the relative frequency of sun exposure between the study participants could explain some of the large variation in the outcome of different clinical trials.

2. Vitamin D independent effects of UVB light - clinical UVB phototherapy trials-

In addition to the health benefits of Vit D synthesis, sunlight also exerts beneficial immunomodulatory effects that are independent of Vit D (for review see (Hart et al., 2011, Lucas et al., 2015, DeLuca and Plum, 2017)) suggesting probable additional Vit D independent effects of UVB exposure in the pathophysiology of MS. Excessive UVB irradiation is known to cause apoptotic cell death. However, UVB light also up-regulates the production of melatonin, which acts as an antioxidant and direct radical scavenger, thereby increasing the survival of epithelial as well as immune cells in an Nrf2-dependent pathway (Janjetovic et al., 2017; Janjetovic et al., 2014). Interestingly, in PBMCs from MS patients melatonin significantly decreased Th1 and Th22 responses (Alvarez-Sanchez et al., 2017). Moreover, the authors demonstrated an up-regulated expression of the melatonin receptor in MS patients compared to healthy volunteers, thus suggesting the melatonin pathway as a Vit D independent effect of UVB light, which is possibly able to, at least partially, explain the molecular mechanisms underlying the immunomodulatory effects of UVB.

Even though it is very challenging, some would probably say impossible, to itemize which processes are mediated via Vit D synthesis and which by UVB light in the human system, there are a few elaborate studies suggesting direct effects of sunlight exposure. An epidemiological study conducted in Australia suggests that sun exposure, measured by actinic damage and self-reported sun exposure, and Vit D status are independent risk factors for CNS demyelination: higher actinic skin damage and higher Vit D levels were independently associated with reduced demyelinating events (Lucas et al., 2011). These findings are supported by a recent study reporting direct effects of sun exposure on MRI measures of neurodegeneration in MS, which appear to be dissociated from elevated Vit D levels that result from increased UVB exposure (Zivadinov et al., 2013). A Swedish epidemiological study also suggests partly different pathways for UVB exposure and Vit D status in mediating beneficial effects against developing MS, as an association between UVB and MS risk persisted after adjustment for serum Vit D levels (Baarnhielm et al., 2012). Along the same lines, another study that was conducted in the North of Scotland and investigated the effects of UVB light therapy on Vit D levels and systemic immune functions in patients with inflammatory skin diseases, suggested that UVB light and Vit D may affect particular immune functions independently from each other (Milliken et al., 2012).

As all these studies propose specific effects of UVB light that are exerted in parallel (or independent) to the immunomodulatory effects of Vit D and in light of a lack of definitive outcomes in MS patients after clinical trials of Vit D supplementation implying that Vit D administration alone might not have the capacity to compensate for insufficient UVB light exposure, investigational trials assessing the benefits of narrowband UVB light therapy on MS development and progression are needed. We have recently conducted a small, interventional proof-of-concept trial investigating the effects of UVB phototherapy in MS patients. Patients were treated five times per week for six weeks with narrowband UVB phototherapy (311 nm), an intervention that is also given to patients with psoriasis and has proven safe and effective (Archier et al., 2012; Paul et al., 2012). The treatment dose was independently developed to suit each participant's skin type and the therapy resulted in higher Vit D levels and immunomodulatory effects in the skin as well as in peripheral blood. However, our trial was not powered to observe effects on clinical outcomes (Breuer et al., 2014). To our knowledge, there are currently 2 phototherapy trials recruiting participants, which will hopefully help to decipher Vit D dependent and independent benefits of UVB exposure:

- **PhoCIS** ("Narrow band ultraviolet-B (UVB) light for patients with

clinically isolated syndrome"; ACTRN12614000185662; interventional, randomized trial) tests whether a course of narrow band UVB phototherapy decreases the risk of developing MS in subjects who have experienced a first demyelinating event. An estimated 60 individuals diagnosed with CIS within the last 120 days, of which 30 will be randomized to the treatment group and 30 to the control group, will be recruited. As a standard care, all participants will receive sufficient Vit D to achieve serum Vit D levels of 80 nmol/l and subjects assigned to the treatment group will in addition receive a total of 24 narrowband UVB phototherapy (311 nm) administrations over 8 weeks. Study participants will be neurologically and immunologically assessed for 12 months and effects of UVB therapy will be analyzed on immune and inflammatory markers of disease, as well as on MRI (Hart et al., 2017). Study participants are currently recruited and so far at least 18 patients with CIS have been included into the study (Jones et al., 2017). The additional value of the **PhoCIS** trial in comparison to the **PrevANZ** and **D-Lay-MS** trial, which are both investigating the effects of Vit D supplementation on the risk of developing MS after a first demyelinating event, will be that participants who receive narrowband UVB phototherapy gain in addition to Vit D the benefits of other molecules implicated in UVB-induced immunoregulation.

Another clinical trial ("**Phototherapy in persons with multiple sclerosis**"; NCT02365259) examining the effect of narrowband UVB phototherapy on Vit D synthesis in persons with MS is reported to be underway. However, the recruitment status of the trial is unknown and results are not yet available. Nevertheless, this study would help to evaluate to which extent serum Vit D levels in MS patients could be raised by narrowband UVB therapy.

A possible link between sunlight exposure and MS risk might also be the neuropeptide alpha-melanocyte-stimulating hormone (α-MSH), which can be released upon UVB radiation by proteolytic cleavage of its precursor proopiomelanocortin (POMC; (Schiller et al., 2004)). Aside from its originally identified role in skin tanning, α-MSH has been shown to have anti-inflammatory effects and to exert protective immunomodulatory activities via the melanocortin receptor MC1R, which can also be expressed on immune cells, in mouse models of colitis, psoriasis or rheumatoid arthritis (Luger and Brzoska, 2007; Maaser et al., 2006; Auriemma et al., 2012). Moreover, a recent study by Mykicki et al. (Mykicki et al., 2016) used a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), to demonstrate long-lasting, direct neuroprotective effects of NDP-MSH, a stabilized derivative of α-MSH. In isolated hippocampal neurons NDP-MSH was able to, at least partially, recover neuronal function by restoring action potential firing after excitotoxic stress (Mykicki et al., 2016). Importantly, due to its size and chemical structure NDP-MSH, in contrast to many other drugs used in MS therapy, penetrates the blood-brain barrier and moreover, has recently been EMA-approved for the treatment of porphyria, an inherited disorder in heme biosynthesis. Hence, this compound (Scenesse®) might represent a perfect possibility for drug repurposing. Scenesse®, based on our in vitro data and the observations made in mice models (Mykicki et al., 2016), could be an interesting and promising target for further clinical development in MS therapy. Thus, the characterization of the external factor UVB light as potent immunomodulator in MS (Breuer et al., 2014) and the identification of a possible mechanism by which external signals might be transmitted into susceptible organisms (Mykicki et al., 2016) followed by the identification of approved drugs that could be repurposed (Scenesse®) might represent an innovative concept of using environment mimetics for the development of novel MS therapeutics.

3. Conclusions

While sunlight and downstream factors such as Vit D comprise strong evidence for its association with MS susceptibility and disease activity, data are not enough yet to strongly support a significant disease modifying relevance of isolated Vit D supplementation in existing MS. Nevertheless, considering the amount of data suggesting beneficial actions of Vit D and the relatively low risk of side effects (with close monitoring), the usage of Vit D supplements at moderate doses might be a reasonable choice for individuals i) with low Vit D levels and ii) as supplementary alimentation to a given disease modifying therapy. As data are not strong enough to consider Vit D as a monotherapy for prevention or disease modification of existing MS and further evidence is needed to determine whether UVB light exerts beneficial effects on MS independently of Vit D, moderate sun exposure, balanced between the beneficial (e.g. Vit D synthesis; suppression of autoimmune diseases) and detrimental (e.g. increased risk of skin cancer) effects of UVB light exposure, as well as a diverse diet including Vit D precursors, is highly recommended.

Box 1

Background - Vitamin D source and metabolism.

Vitamin D is a secosteroid hormone, which can be ingested from food, but the main source of vitamin D in humans is skin exposure to sunlight with > 80% of vitamin D being synthesized in the skin, a process that depends particularly on active wavelengths of ultraviolet B (UVB) (290–315 nm) (Hart and Gorman, 2013). Upon exposure to solar UVB radiation 7-dehydrocholesterol, the precursor of vitamin D in keratinocytes, is converted to pre-vitamin D_3, which then immediately isomerizes into vitamin D_3 in a heat-dependent process (Holick et al., 2007). Vitamin D_3 from the skin and from dietary sources undergoes then two successive hydroxylation steps to form the active molecule 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. The first activation step takes place in the liver, where vitamin D_3 is hydroxylated by the enzyme 25-hydroxylase (CYP27A1) to 25-hydroxy vitamin D_3 [$25(OH)D_3$], which is the major circulating metabolite of vitamin D_3. In the second hydroxylation step $25(OH)D_3$ is metabolized to its active form $1,25(OH)_2D_3$. This final hydroxylation occurs in the kidney and is catabolized by the enzyme 25-hydroxyvitamin D_3–1α-hydroxylase (CYP27B1) (Mora et al., 2008; Hart et al., 2011; Bouillon et al., 2008).

The major pathway for the synthesis of the active form of vitamin D as described above involves liver and kidney; however, the complete pathway can also be achieved locally in the skin as keratinocytes possess the enzymatic machinery to metabolize vitamin D (Lehmann et al., 2003; Bikle et al., 1986). Vitamin D can further be metabolized by cells of the immune system, since multiple cell types express the enzyme CYP27B1, allowing them to synthesize $1,25(OH)_2D_3$ from circulating $25(OH)D_3$ (Sigmundsdottir et al., 2007; Holick, 2007).

Finally, $1,25(OH)_2D_3$ is catabolized by the enzyme 24-hydroxylase (CYP24A1) to calcitroic acid, its inactive metabolite, that is then excreted in the bile (Mora et al., 2008).

$25(OH)D_3$, the major circulating form of vitamin D, is used by clinicians to determine the vitamin D status. Although there is no consensus on optimal levels of circulating $25(OH)D_3$, levels of < 50 nmol/l (20 ng/ml) are generally regarded as insufficient and levels of < 25 nmol/l (10 ng/ml) as deficient. A level of 75 nmol/l (30 ng/ml) or greater on the contrary is considered as optimal for good health. In the case of vitamin D intoxication serum levels > 374 nmol/l (150 ng/ml) are observed (Holick, 2007).

In order to achieve sufficient levels of circulating $25(OH)D_3$ by sun exposure, often 5 to 30 min of sunlight exposure are adequate. However, this depends on variables that relate to

each individual (skin pigmentation, area of sun-exposed skin, baseline levels of circulating 25(OH)D_3) and to environmental factors that influence the intensity and spectral range of UVB light in the environment (time of the day, season, latitude) (Holick et al., 2007; Hart et al., 2011).

Declaration of interest

K.L. is coinventor on patent no. US 2016/0158322 A1 entitled NDP-MSH for treatment of inflammatory and/or neurodegenerative disorders of the CNS. The other authors declare no competing interests.

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