Supplementary Information for

Sunlight exposure exerts immunomodulatory effects to reduce multiple sclerosis severity

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KKNMS and BIONAT study investigators can be found in the appendix

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This file includes:

- Supplementary methods
- Supplementary Figure S1
- Supplementary Tables S1-S4
- Supplementary references
- Study group members for the KKNMS (NationMS) and BIONAT cohorts

Other supplements include:

- Supplementary Datasets S1-10 (xlsx-files)

Supplementary methods

Disease severity outcomes

The multiple sclerosis severity score (MSSS) was used as the primary measure of disability in both cohorts. The MSSS was calculated from the recorded expanded disability status scale (EDSS) values according to Roxburgh (1). Additionally, for the *NationMS* cohort, magnetic resonance imaging (MRI) was carried out according to standardized protocols (2) and assessed for gadolinium (Gd)-enhancing lesions in T1-weighted images. Electromagnetic Field strength was 3 Tesla across all centers and evaluation was carried out at each site separately by certified neuro-radiologists. To assess disability accumulation over time in the *NationMS* cohort, the change in the EDSS was assessed between follow up 2 (T2, 2 years from baseline) and baseline visit. Furthermore, relapses during the course of the study were recorded. A relapse was defined according to the criteria of the German Neurological Society (DGN). Briefly, the occurrence (or re-occurrence) of clinical symptoms that do not resolve within 24 hours with no previous relapse in the last 30 days and that cannot be explained by acute infections were regarded as a relapse.

Estimation of ambient ultraviolet radiation using satellite data

Ultraviolet radiation (UVR)-exposure was estimated to parallel the analysis of association between latitude and disease severity and to determine the influence of recent UVR exposure on serum vitamin D (vitD) levels. To generate a variable that estimates available UVR at the locations of the medical centers, data from NASA's (National Aeronautics and Space Administration) well-validated ozone monitoring instrument (OMI) dataset were used (3, 4). The OMI datasets comprises records of local noon time erythemal UV doses with a resolution of one degree in both latitude and longitude. UVR exposure one year before blood draw for patients of the *NationMS* and the *BIONAT* cohort was estimated by accumulating the daily erythemal doses 365 days before blood draw / medical assessment at the location of the patient's medical center. The time span of one year has been chosen to achieve coordinate-specific estimates that are not biased by season. Notably, it was not possible to generate lifetime exposure estimates, as a record of residence was not available for most of the patients. Furthermore, to rule out that estimates were biased by patients who had moved in the year before study inclusion, a sub-group analysis of 216 patients for whom residency information was available was performed in the *NationMS* cohort. Ten patients reported to have moved in the year before inclusion. Nine of these ten patients moved from places nearby their current residence (<100 km). Assuming a similar behavior for the remaining patients, estimates (latitude or satellite-derived UVR estimates) should not be heavily biased by patients, who have moved in the year before inclusion. Parameters from statistical models using the UVR estimates are provided according to an increase of 1 W/m².

RNA sequencing analysis

RNA-sequencing (RNAseq) was performed using samples of five patients from our 2014 pilot study. Phototherapy was carried out in November to March to rule out sources of external UVR exposure (5).

Frozen PBMC samples from before and after four weeks of phototherapy (ten samples in total) were thawed and stained for CD3, CD4, CD8, CD14, CD19 and CD56 (all antibodies from Biolegend). Viable cells were identified by forward and sideward scatter characteristics. T-cell subsets were identified as CD56-CD3+ cells being either CD4+CD8- or CD8+CD4- cells. B-cells were identified as CD3-CD56-CD14-CD19+, and monocytes were identified as CD3-CD19-CD56-CD14+ cells. Cells were sorted on a FACS Aria III machine and subjected to RNA isolation. RNA sequencing was performed on an Illumina NextSeq 500. QC was done using *FastQC* and read quantification was done in *Kallisto (6)*. Differential gene expression analysis was performed using the quasi-likelihood approach in *edgeR (7)*. To test the enrichment of the vitD- and type I interferon-associated genes, distribution-free permutation tests were performed (8). Due to the limited sample size and the exploratory nature of this part of the study, genes with *P*-values < 0.05 were used for enrichment tests. Genes associated with the respective pathways were extracted from *wikipathways* (9), collapsing the quality approved gene-/protein lists from the pathways 'Vitamin D receptor-pathway' (WP2877) and 'Non-genomic effects of vitamin D' (WP4341) to generate a reference set of vitD-associated genes. For the type I interferon pathway, the gene-/protein lists from the 'Type I interferon signaling pathway' (WP585) and the 'DDX58/IFIH1-mediated induction of interferon-alpha/beta'-pathway (WP1904) were collapsed to generate a reference set of type I interferon-associated genes. For permutation tests, the overlap of genes regulated by phototherapy ($P<0.05$) for each respective cell type with the reference gene-set was calculated. Next, random samples of the same size as the list of genes with $P<0.05$ were taken from the list of all genes expressed by the respective cell type, and the overlap with the reference gene-set was calculated. To obtain an approximate *P*-value the random sampling was repeated 10,000 – 1,000,000 times and the number of occasions when randomly sampled genes overlapped equally or more than the test-set with the reference sets, was divided by the number of permutations (8).

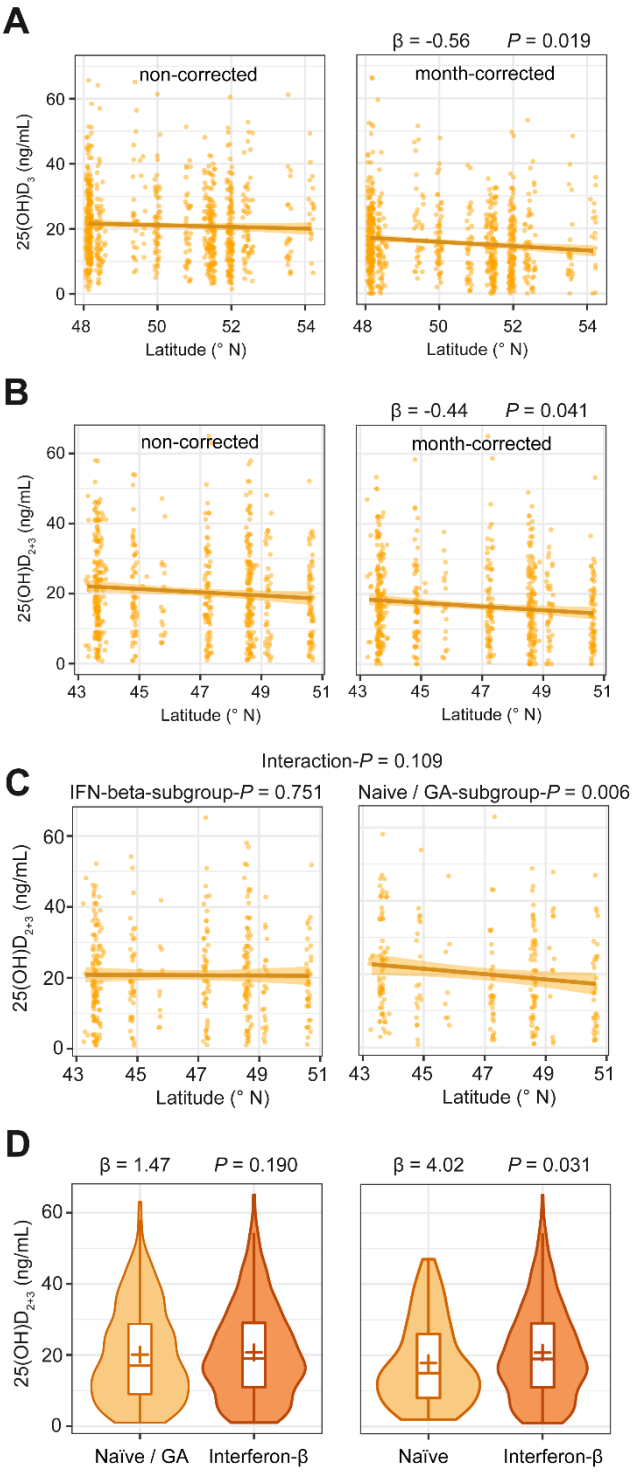

Figure S1: Effect latitude and medication on serum vitamin D

A & B Dot-plots showing the influence of latitude on serum vitamin D with a least-squares regression line ± standard error for **A** the *NationMS* cohort and **B** the *BIONAT* cohort. Values in the right panels were corrected for sampling month by first regressing sampling month on serum vitamin D and using the model residuals plus the model intercept as month-corrected vitamin D levels. **C** Dot plots showing the effect of latitude on serum vitamin D levels if stratified by prior treatment in the *BIONAT* cohort (IFN-beta-treated vs. untreated or Glatiramer acetate (GA)-treated) with least-squares regression lines ± standard error. **D** Violin-plot showing serum vitamin D-levels with regard to prior medication for the *BIONAT* cohort.

Table S1: Cohort baseline characteristics

	NationMS	***BIONAT***
Number of patients	908	808
Median Age (IQR)	32.43 (26.76 to 41.15)	37 (30 to 43)
Median BMI (IQR)	24.22 (21.56 to 27.64)	-
Male (%)	271/908 (29.85)	196/808 (24.26)
Smokers (%)	305/908 (33.59)	-
Consume alcohol (%)	674/908 (74.23)	-
RRMS (%)	485/908 (53.42)	808/808 (100)
Mean 25(OH)D[a] (SD)	21.56 ng/mL (12.87)	20.95 ng/mL (14.23)
Median MSSS (IQR)	4.31 (2.83 to 5.58)	5.23 (3.41 to 6.92)
≥ 1 Gd-enhancing lesion (%)	350 (38.55)	-
Most common site / symptom of first manifestation[a] (%)	Sensory system (411/908, 45.26%)	Spinal cord (138/611, 22.59 %)

Abbreviations: 25(OH)D = 25-hydroxy-vitaminD, BMI = body mass index (weight/height²), Gd = Gadolinium, IQR = interquartile range, MSSS = multiple sclerosis severity score, RRMS = relapsing-remitting multiple sclerosis. [a] According to the number of patients with available data (see Figure 1)

Table S2: Medication before or after first assessment

NationMS		
Medication after first assessment	**Number**	**Percent (of 798 total)**
Fingolimod	21	2.63
Fumarat	64	8.27
Glatiramer acetate	162	20.30
Interferon-β	364	45.61
Monoclonal antibodies[a]	23	2.88
Naïve	148	18.54
Teriflunomide	14	1.75
BIONAT		
Medication before first assessment	**Number**	**Percent (of 808 total)**
Glatiramer acetate	236	29.21
Interferon-β	483	59.78
Naïve	89	11.01

[a] The monoclonal antibodies Natalizumab, Rituximab and Alemtuzumab were grouped due to low frequencies

Table S3: Results for the effect of satellite-estimated UVR with or without interactions

Cohort	Dependent	Interaction or subgroup	Model	Estimate[a]	95% CI	*P*-value
NationMS	MSSS	-	LM	-0.0048	-0.0085 to -0.0011	0.009
	Gd+ lesions	-	NB-GLM	0.997	0.994 to 1.001	0.137
	Relapses	-	Cox	1.001	0.998 to 1.003	0.565
	ΔEDSS	-	LM	-0.0018	-0.004 to -0.003	0.023
	Gd+ lesions	rs1805008	NB-GLM	1.02	1.01 to 1.029	0.00005
	Gd+ lesions	rs1805008:CT/TT-subgroup	NB-GLM	1.015	1.004 to 1.035	0.0034
	Gd+ lesions	rs1805008:CC-subgroup	NB-GLM	0.994	0.982 to 0.998	0.0068
BIONAT	MSSS	-	LM	0.0003	-0.0016 to 0.002	0.785
	MSSS	Treatment (IFN-beta vs. Untreated / GA)	LM	-0.0036	-0.0073 to 0.00001	0.051
	MSSS	IFN-beta-subgroup	LM	0.0015	-0.0009 to 0.0039	0.237
	MSSS	Untreated / GA-subgroup	LM	-0.0026	-0.0554 to 0.0003	0.080

Abbreviations: CI = Confidence interval, Gd = Gadolinium, GA = Glatirameracetate, EDSS = expanded disability status scale, IFN-beta = interferon-beta, LM = linear model, MSSS= Multiple sclerosis severity score, NB-GLM = negative-binomial generalized linear model. [a] For linear models regression coefficients are provided, for cox regressions hazard ratios are provided and for negative-binomial generalized linear models risk ratios are provided. All models are adjusted as the models used for the analysis of association between latitude and the dependent variables. Model parameters are provided according to an increase in the sum (1 year) of estimated UVR of 1 W/m².

Table S4: Site or Symptom of first manifestation

NationMS[a]		
Site / Symptom	**Number**	**Percent (of 908 total)**
Bladder	3	0.3
Brainstem	81	7.8
Cerebellum (tremors, ataxia, etc.)	42	4.6
Depression / cognitive dysfunctions	7	0.7
Motor dysfunctions	40	4.4
Other	10	1.1
Sensory deficit	411	45.3
Vision disorder	314	34.6
BIONAT[a]		
Site / Symptom	**Number**	**Percent (of 611 total)**
Acute disseminated encephalomyelitis	1	0.16
Cerebral Hemisphere	15	2.45
Facial sensory system	1	0.16
Multifocal lesions	59	9.66
Oculomotor	1	0.16
Optic Neuritis	129	21.11
Posterior Fossa	123	20.13
Pyramidal syndrome	32	5.24
Sensory deficit	111	18.17
Spinal cord	138	22.59
Walking difficulties	1	0.16

[a] Definitions of first sites / symptoms were different across cohorts

Supplementary references:

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