

Electronic Supplementary Information

Pigment genes not skin pigmentation affect UVB-induced vitamin D

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Table S1 UVB-doses and 25(OH)D levels during study period

Study Day	UVB per week in kJ m ⁻² (SED)	Mean 25(OH)D levels (nmol l ⁻¹) in \pm SD (range)
0	-	67 \pm 27 (10-120)
7	1.9 (4)	77 \pm 24 (32-143) ^a
13	1.9 (4)	85 \pm 23 (42-143) ^b
20	2.8 (6)	88 \pm 24 (45-147)
28	2.8 (6)	94 \pm 26 (50-175) ^b
34	2.8 (6)	99 \pm 27 (54-179) ^b
41	2.8 (6)	104 \pm 27 (57-178) ^b
48	2.8 (6)	107 \pm 28 (62-184)
55	4.2 (9)	112 \pm 32 (63-196) ^b
62	4.2 (9)	118 \pm 32 (73-216) ^b

Days are given as mean study days. ^a Significant ($P < 0.05$) increase in 25(OH)D from this point and forward compared to Day 0. ^b Significant ($P < 0.05$) increase in 25(OH)D compared to immediately preceding sample point.

Table S2 Separate influence of demographic parameters on the slope of the UVB induced increase of 25(OH)D

Model / Parameters	R ²	P-value	Power
GLM (individual intercepts, common slope)	0.629	2.7×10^{-86}	1.000
Facultative PPF	0.655	8.0×10^{-8}	1.000
Constitutive PPF	0.648	8.2×10^{-6}	0.995
Height	0.650	2.5×10^{-6}	0.998
Fatty fish intake	0.646	1.8×10^{-5}	0.991
Age	0.643	9.1×10^{-5}	0.976
Sex	0.637	3.4×10^{-3}	0.836
Body surface area	0.636	8.7×10^{-3}	0.748
BMI	0.634	0.022	0.633
Weight	-	0.15	-

A total of 62.9% of the variation in the 25(OH)D increase was explained by a general linear model (GLM) comprising individual (measured) intercepts and a common slope. Additional explanation of the variation was assessed by investigating the influence of different parameters on the variation due to the slope. $P < 0.05$ was considered significant. Power is the probability of confirming the given result in a new material with similar size and uncertainties as this material. R² is squared correlation coefficient. Skin pigment protection factor (PPF) is an objective measurement of skin pigmentation with a measurement range of 1-25. Constitutive PPF measuring site was buttocks. Facultative PPF was a mean of measuring sites on chest, midriff, back of shoulder, medial and lateral sides of arm.

Table S3 Separate influence of SNPs located in pigment genes on the slope of the UVB induced 25(OH)D increase

SNPs/Model	N	R ²	P-value	Power	Allele effect	Cytogenetic localization	Gene localization
GLM with individual intercepts and common slope		0.629	2.7×10 ⁻⁸⁶	1.000			
rs2762464 — no. AT/TT/AA	18 / 13 / 9	0.672	4.5×10 ⁻¹¹	1.000	no	9p23	Tyrosinase-related protein 1
rs1042602 — no. AC/CC/AA	16 / 18 / 6	0.669	2.4×10 ⁻¹⁰	1.000	no	11q14.3	Tyrosinase
rs1042522 — no. CC/CG/GG	10 / 11 / 19	-	2.6×10 ⁻¹⁰	-	no	17p13.1	Tumor protein p53
rs4911442 — no. AG/GG/AA	9 / 0 / 31	0.663	1.1×10 ⁻⁹	1.000	-	20q11.22	Agouti signaling protein
rs28777 — no. AC/CC/AA	8 / 6 / 26	0.666	1.6×10 ⁻⁹	1.000	yes	5p13.2	Solute Carrier Family 45, Member 2
rs16891982 — no. CC/CG/GG	7 / 7 / 26	0.663	8.2×10 ⁻⁹	1.000	yes	5p13.2	Solute Carrier Family 45, Member 2
rs12896399 — no. GG/TG+TT	12 / 22 + 8	0.649	1.6×10 ⁻⁵	0.997	yes	14q32.12	Solute Carrier Family 24, Member 4
rs4778241 — no. AC/CC/AA	11 / 23 / 6	-	4.2×10 ⁻⁵	-	no	15q12-q13	Oculocutaneous albinism, type II
rs6475555 — no. AA+AG/GG	9 + 18 / 13	0.646	9.1×10 ⁻⁵	0.992	yes	9p21.3	Solute Carrier Family 45, Member 2
rs11614913 — no. CC/CT+TT	19 / 16 + 5	0.642	7.8×10 ⁻⁴	0.965	yes	12q13.13	MIR196A29
rs26722 — no. CC/CT/TT	35 / 5 / 0	0.639	1.1×10 ⁻³	0.908	-	5p13.2	Solute Carrier Family 45, Member 2
rs4821767 — no. AC+CC/AA	23 + 10 / 7	0.642	1.2×10 ⁻³	0.919	yes	22q13.1	Phospholipase A2, Group VI
rs4911414 — no. GG/TG+TT	20 / 18 + 2	0.641	1.7×10 ⁻²	0.901	-	20q11.22	Agouti signaling protein
rs3733542 — no. CC+CG/GG	1 + 7 / 32	0.635	1.9×10 ⁻²	0.602	-	4q12	Proto-oncogene receptor tyrosine kinase
rs2284063 — no. AA+AG/GG	18 + 17 / 5	0.637	1.9×10 ⁻²	0.712	yes	22q13.1	Phospholipase A2, Group VI
rs2031526 — no. AA+AG/GG	2 + 12 / 26	0.676	3.1×10 ⁻²	1.000	-	13q32.1	Dopachrome tautomerase
rs2276288 — no. AA+AT/TT	7 + 23 / 10	0.635	3.2×10 ⁻²	0.689	yes	11q13.5	Myosin family 7, member A
rs401681 — no. CC/CT/TT	14 / 18 / 8	-	0.051	-	-	5p15.33	Cisplatin resistance-related like protein 1
rs3212361 — no. CC/CT/TT	19 / 16 / 5	-	0.17	-	-	16q24.3	Melanocortin 1 receptor
rs3212359 — no. AG/GG/AA	15 / 16 / 9	-	0.21	-	-	16q24.3	promotor region Melanocortin 1 receptor
rs1015362 — no. AA+GA/GG	1 + 18 / 21	-	0.22	-	-	20q11.22	promotor region Agouti signaling protein

rs199355 — no. CC/CT+TT	19 / 17 + 4	-	0.33	-	-	11q14.3	Tyrosinase
rs12203592 — no. CC/CT+TT	35 / 4 + 1	-	0.47	-	-	6p25.3	Interferon regulatory factor 4
rs11636232 — no. CC/CT/TT	19 / 16 / 5	-	0.62	-	-	15q13.1	E3 ubiquitin- protein ligase
rs8059973 — no. AG/GG/AA	13 / 27 / 0	-	0.62	-	-	16q24.3	Dysbinding domain containing 1
rs12913832 — no. AG/GG/AA	8 / 17 / 15	-	0.68	-	-	15q13.1	E3 ubiquitin- protein ligase
rs1393350 — no. AG/GG/AA	12 / 28 / 0	-	0.68	-	-	11q14.3	Tyrosinase
rs12623857 — no. AA+AG/GG	1 + 8 / 31	-	0.69	-	-	2q36.1	Paired box gene 3
rs1126809 — no. CC/CT/TT	28 / 12 / 0	-	0.79	-	-	11q14.3	Tyrosinase
rs7495174 — no. AG/GG/AA	8 / 1 / 31	-	0.88	-	-	15q12-q13	Oculocutaneous albinism, type II
rs258322 — no. CC/CT/TT	36 / 4 / 0	-	-	-	-	16q24.3	Cyclin- dependent kinase 10

A total of 62.9% of the variation in the 25(OH)D increase was explained by a general linear model (GLM) comprising individual intercepts (measured) and common slope. Additional explanation of the variation was assessed by investigating the influence of SNPs located in genes with influence on pigmentation in this model. A SNP allele frequency of at least 5 in each subgroup was retained. SNPs not displaying allele effect (allele dose effect or dominant allele effect) were excluded. $P < 0.05$ was considered significant. R^2 is squared correlation coefficient. Power is the probability of confirming the given result in a new material with similar size and uncertainties as this material.

Table S4 Relation between measured skin pigmentation and pigmentation SNPs. The influence of SNP subgroups on the level of skin pigmentation is shown in the table.

SNP	Frequency	Facultative PPF	P-value	Constitutive PPF	P-value
<u>Pigment SNPs with influence on 25(OH)D increase in a combined GLM</u>					
rs28777			7.2×10^{-8}		7.5×10^{-9}
CA	8	8.4		6.7	
CC	6	12.4		12.6	
AA	26	6.8		4.2	
rs16891982			1.0×10^{-6}		4.2×10^{-4}
CC	7	11.8		11.6	
CG	7	8.4		6.9	
GG	26	6.8		4.2	
rs11614913			0.026		0.046
CC	19	8.9		7.2	
CT+TT	21	7.1		4.9	
rs4911442			0.37 (ns)		0.052 (ns)
AG	9	7.3		3.9	
AA	31	8.2		6.6	
rs4911414			0.29 (ns)		0.42 (ns)
GG	20	8.4		6.5	
GT+TT	20	7.5		5.5	
rs12896399			0.39 (ns)		0.23 (ns)
GG	12	8.5		7.1	
TT+GT	28	7.7		5.5	
rs6475555			0.43 (ns)		0.70 (ns)
AA+AG	27	7.7		5.8	
GG	13	8.4		6.3	
<u>Pigment SNPs without influence on 25(OH)D increase in a combined GLM</u>					
rs11636232			0.025		4.2×10^{-4}
CC	19	9.1		8.2	
CT	16	7.0		3.9	
TT	5	6.9		4.0	
rs12913832			0.026		0.0019
AG+GG	25	7.1		4.3	
AA	15	9.5		8.7	
rs2276288			0.034		0.15 (ns)
TT	10	6.5		4.5	
AA+AT	30	8.5		6.5	
rs4778241			0.053 (ns)		0.035
AC	11	7.0		5.2	
CC	23	7.8		5.4	

AA	6	10.1	9.5	
rs1042522			0.072 (ns)	0.011
CC	10	9.3	8.9	
CG	11	8.3	5.5	
GG	19	7.0	4.7	
rs3212359			0.28 (ns)	0.25 (ns)
AA+AG	24	8.3	6.5	
GG	16	7.4	5.1	
rs3212361			0.24 (ns)	0.21 (ns)
TT	5	9.2	7.9	
CT + CC	35	7.8	5.7	
rs1042602			0.16 (ns)	0.12 (ns)
CC	18	9.1	7.0	
CA + AA	22	7.1	5.1	
rs1126809			0.13 (ns)	0.076 (ns)
CC	28	8.4	6.7	
CT	12	7.0	4.4	
rs1015362			0.73 (ns)	0.29 (ns)
AA+AG	19	8.4	5.4	
GG	21	7.5	6.6	
rs2031526			0.17 (ns)	0.48 (ns)
AA+AG	14	7.2	5.4	
GG	26	8.4	6.3	
rs3733542			0.39 (ns)	0.52 (ns)
CC+CG	8	8.7	6.8	
GG	32	7.8	5.8	
rs12203592			0.47 (ns)	0.34 (ns)
CC	35	8.1	6.2	
CT+TT	5	7.2	4.5	
rs199355			0.98 (ns)	0.85 (ns)
CC	19	8.0	5.9	
CT+TT	21	8.0	6.1	
rs1393350			0.17 (ns)	0.12 (ns)
AG	12	7.1	4.6	
GG	28	8.3	6.6	
rs8059973			0.46 (ns)	0.35 (ns)
AG	13	7.5	5.2	
GG	27	8.2	6.4	
rs12623857			0.86 (ns)	0.24 (ns)
GG	31	8.0	6.4	

AA+AG	9	7.8	4.7
rs2284063		0.20 (ns)	0.14 (ns)
AG+GG	22	7.5	5.2
AA	18	8.5	6.9
rs2762464		0.069 (ns)	0.034 (ns)
AT	18	8.2	6.7
TT	13	6.8	3.8
AA	9	9.3	7.5
rs4821767		0.40 (ns)	0.39 (ns)
AC	23	8.0	5.9
CC	10	8.6	7.1
AA	7	6.9	4.6
rs401681		0.38 (ns)	0.24 (ns)
CC	14	8.5	6.9
CT+TT	26	7.7	5.5
rs7495174		0.83 (ns)	0.14(ns)
AG+GG	9	8.1	7.6
AA	31	7.9	5.5
rs26722		0.14 (ns)	0.22 (ns)
CC	35	7.7	5.7
CT	5	9.6	7.9

Skin pigment protection factor (PPF) is an objective measurement of skin photo-type with a measurement range of 1-25. Mean values are given. Constitutive and facultative PPF measuring sites were buttock and a mean of chest, midriff, back of shoulder, medial and lateral sides of arm, respectively. $P < 0.05$ was considered significant. Significant differences in PPF of SNPs with 2 and 3 subgroups were investigated with general linear models. Non-significant influence was indicated with (ns).

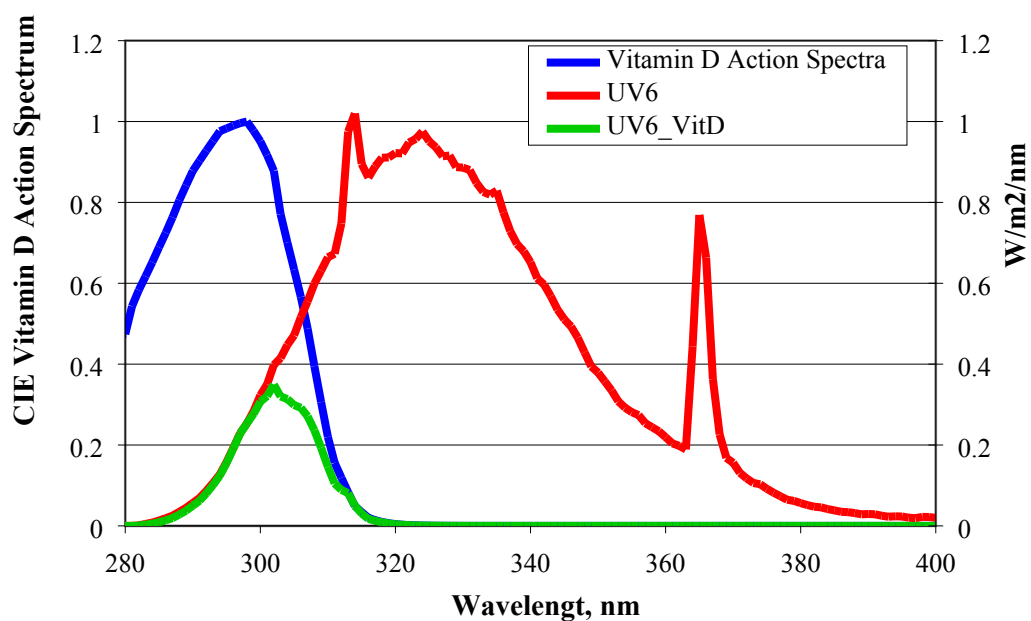


Fig. S1 The UV spectrum for a F85/100W UV6 lamp. The blue line represents action spectrum of the production of pre-vitamin D₃ in humans (CIE 174 Technical Report, 2006). The red line represents the emission spectrum for the UV6 lamp (broadband UVB, 290-360 nm). The green line is the CIE pre-vitamin D₃ weighted UV action spectrum of the UV6 lamp, of which 90% (295-360 nm) is present in daylight during a summer day in Denmark.

SNP typing

The samples were typed for 31 SNPs located in genes potentially influencing pigmentation. This was performed using the iPLEX[®] Gold kit (Sequenom). The PCR contained 2 μ L DNA, 0.5 μ L 10x buffer, 0.8 μ L 25mM MgCl₂, 0.1 μ L 25mM dNTP mix, 1.30 μ L 0.5 μ M primer (Table S1) mix (DNA Technology), 0.2 μ L 5U/ μ L HotStarTaq, and 1.1 μ L H₂O. The PCR was performed in a GeneAmp[®] PCR system 9700 thermal cycler (Thermo Fisher Scientific) with the following conditions: denaturation at 94°C for 2 min followed by 45 cycles of 94°C for 20 s, 62°C for 30 s, 72°C for 1 min, followed by 72°C for 3 min. The PCR products were treated with Shrimp Alkaline Phosphatase (SAP) (Sequenom) in a GeneAmp[®] PCR system 9700 thermal cycler (Thermo Fisher Scientific) at 37°C for 40 min and 85°C for 5 min. The SBE reaction contained 8 μ L SAP treated PCR products and 2 μ L iPLEX[®] mix (Sequenom). The iPLEX[®] mix contained 0.2 μ L 10x iPLEX[®] buffer, 0.2 μ L iPLEX[®]-Termination mix, 0.94 μ L primer mix (DNA Technology), 0.04 μ L iPLEX[®]-enzyme, and 0.62 μ L H₂O. The SBE reaction was performed in a GeneAmp[®] PCR system 9700 thermal cycler (AB) with the following conditions: denaturation at 94°C for 30 s followed by 40 cycles of 94°C for 5 s, 52°C for 5 s, 80°C 5s, 52°C for 5 s, 80°C for 5 s, 52°C for 5 s, 80°C for 5 s, 52°C for 5 s, 80°C for 5 s, 52°C for 5 s, 80°C 5s, followed by 72°C for 3 min.

A total of 40 μ L of molecular grade water and ion exchange resin (Sequenom) was added to each sample. The samples were rotated for approximately 4h and kept in the refrigerator for up to 4 days before spotting. The samples were spotted using the RS1000 Nanospotter (Sequenom) and analysed on the MassARRAY[®] Analyzer 4 System (Sequenom) using the auto-run settings. The samples were analysed with Typer Analyzer 4 (Sequenom) and were auto-clustered using a signal to noise ratio of at least seven. Cluster plots were visually inspected and outliers were further investigated. All samples were investigated in duplicate. The genotypes were compared between duplicate spotting and typing results using a custom made script (PlateCompare) developed with the statistic software R (R core team, version 2.11.0, URL

<http://www.R-project.org>). Further details on the SNP typing method are described elsewhere.¹⁻⁴ The genetic analyses were performed between 2011 and 2017.

References

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	Primer2	ACGTTGGATGGGAAGGTGAATGATAACACG
	SBE primer	TCCCTTCTCTGCAAC
rs8059973	Primer1	ACGTTGGATGGGCAGGTGGTTCTGTGTAA
	Primer2	ACGTTGGATGTTCCCGAGTAGCTGCCACAC
	SBE primer	GGGTCTGTGTTAATAATGACAGCT
rs6475555	Primer1	ACGTTGGATGGAAAGAAGTACACCAGTCC
	Primer2	ACGTTGGATGGAATGAGTTAGGGAGGATGC
	SBE primer	CATCCTACTGAAACTATTCC
rs1042522	Primer1	ACGTTGGATGAGACCCAGGTCCAGATGAAG
	Primer2	ACGTTGGATGGGTGTAGGAGCTGCTGGTG
	SBE primer	CGCCAGAGGCTGCTCCCC
rs2276288	Primer1	ACGTTGGATGATGGTGACAGTGGGCATGAC
	Primer2	ACGTTGGATGGCCAACGGCATCAATGAGAG
	SBE primer	TAAAGTGGGCATGACGTACACAC
rs12623857	Primer1	ACGTTGGATGCTTCCCACAGTGTCCACTC
	Primer2	ACGTTGGATGGCAGCGGCCTGCCGTTGAT
	SBE primer	CCTCAACCAGCTCGGCGG
rs2284063	Primer1	ACGTTGGATGTGCCTGATTACACTTTCCCC
	Primer2	ACGTTGGATGAGGAGTTTTGCTGTGGTGAG
	SBE primer	CATTAAACTTTCCCATTTCGCTA
rs258322	Primer1	ACGTTGGATGGAAACCTGTTACAGGAACTTC
	Primer2	ACGTTGGATGACCGGATGCTTACGTTTACC
	SBE primer	TTCATGATGTAGTTATCACAAC
rs2762464	Primer1	ACGTTGGATGCTGTTGACAAATATCCATGA
	Primer2	ACGTTGGATGCTTAAAATACATGAATGGGC
	SBE primer	AAATATCCATGAATGGAAATG
rs12896399	Primer1	ACGTTGGATGTCTGGCGATCCAATTCTTTG
	Primer2	ACGTTGGATGAGGAAGTTAATCTGCTGTG
	SBE primer	GTAGGTCAGTATATTTTGGG
rs11614913	Primer1	ACGTTGGATGGGTAGTTTCATGTTGTTGGG
	Primer2	ACGTTGGATGTGACGAAAACCGACTGATG
	SBE primer	TCGGCAACAAGAAACTG
rs4821767	Primer1	ACGTTGGATGTTCTACAGCCAAGTGGAAAC
	Primer2	ACGTTGGATGTGGGAAAGCCACTCTCCCT
	SBE primer	CTCCATCCAAGTGGAAACTATCTT
rs401681	Primer1	ACGTTGGATGGCCAGAAAGCTGCTTCACAC
	Primer2	ACGTTGGATGATGCATAGTGGGCAGAAAAC
	SBE primer	TATCGGCTTCACACCATGAT
rs4778241	Primer1	ACGTTGGATGTGCAATTGTTGGCTGGTAGT
	Primer2	ACGTTGGATGAAATTGTACAGCCACTCTGG
	SBE primer	TGCTGGTAGTTGCAATT
rs7495174	Primer1	ACGTTGGATGTTAGGAAGCAAGGCAAGTTC
	Primer2	ACGTTGGATGTAGGTCGGCTCCGTCGCAC
	SBE primer	CCTTAAGTTCCCCTAAAGGT
rs26722	Primer1	ACGTTGGATGTTGCCAGCTCTGGATTTACG
	Primer2	ACGTTGGATGGTCATCAGATGGAATGTACG
	SBE primer	CCGTAACCATTTTAACTTTCT

Abbreviations: SNP, single nucleotide polymorphism; SBE, single base extension; A, adenosine; C, cytosine; G, guanine; T, thymine.