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Vitamin D in Type 2 Diabetes: Genetic Susceptibility and the Response to Supplementation

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ABSTRACT

Variants of vitamin D metabolism-genes may predispose to type 2 diabetes (T2D). This study investigated the impact of these variants on disease susceptibility, Vitamin D, parathyroid hormone, C-peptide and HbA1c levels before and after cholecalciferol supplementation in patients with T2D. Twelve polymorphisms within CYP2R1, CYP27B1, DBP, VDR and CYP24A1 were genotyped in 553 T2D patients and 916 controls. In addition 65 patients receiving either cholecalciferol or placebo were analyzed during 6 months intervention and 6 months follow-up. T2D risk alleles are VDR rs7975232 "G" ($p_c = 0.031$), rs1544410 "G" ($p_c = 0.027$) and CYP2R1 rs10741657 "A" ($p_c = 0.016$). Patients with genotypes CYP27B1 rs10877012 "CC" ($p_c = 4x10^{-5}$), DBP rs7041 "GG" ($p_c = 0.003$), rs4588 "CC" ($p_c = 3x10^{-4}$), CYP24A1 rs2585426 "CG" ($p_c = 0.006$) and rs2248137 "CG" ($p_c = 0.001$) showed lower $25(OH)D_3$ and DBP rs4588 "CC" lower $1,25(OH)_2D_3$ levels ($p_c = 0.005$). Whereas DBP rs4588 "CC" ($p_c = 0.009$), CYP27B1 rs10877012 "AC" ($p_c = 0.059$), VDR rs7975323 "AG" ($p_c = 0.033$) and rs1544410 "GG" ($p_c = 0.013$) are associated with higher $25(OH)D_3$ levels at 6 months' follow-up. Significant PTH suppression was detected for CYP2R1 "AG" ($p_c = 0.002$), DBP rs4588 "CC" ($p_c < 0.001$), VDR rs110735810 "CT" ($p_c < 0.001$) and CYP24A1 rs2248137 "GG" ($p_c = 0.021$). Genetic variants of the vitamin D system predispose to type 2 diabetes and regulate – partially - vitamin D metabolism, concentrations and the vitamin D status. Vitamin D insufficiency is a T2D risk factor. The response to cholecalciferol supplementation can be measured as $25(OH)D_3$ increment and PTH suppression. This process is regulated by genes of the vitamin D system conferring modest T2D risk.

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Introduction

Vitamin D (VD) insufficiency impairs glucose homeostasis and confers susceptibility to type 2 diabetes (T2D) [1]. The VD status reflects endogenous synthesis via UVB irradiation, dietary intake, and genetic background [2]. The liver enzyme CYP2R1 25-hydroxylase converts vitamin D_3, obtained from previtamin D_3 isomerization, into $25(OH)D_3$, which is the major circulating VD metabolite and indicates the VD status [3]. Circulating VD metabolites are mainly bound to vitamin D binding protein (DBP, also known as GC – group-specific component). The D_3-1α-hydroxylase (CYP27B1) catalyzes the activation to $1,25(OH)_2D_3$ in the kidney and macrophages [4]. $1,25(OH)_2D_3$, activates the vitamin D receptor (VDR), which regulates the expression of genes with a vitamin D response element [5]. Finally, VD is degraded via 24-hydroxylation catalyzed by 25-hydroxyvitamin D 24-hydroxylase (CYP24A1) [6].

Besides environmental factors also genetic variation in the VD system as defined by single nucleotide polymorphisms (SNPs) influences VD serum levels [7].

The purpose of this study was to investigate VD system SNPs in T2D patients, whether they specifically regulate the basal VD status and its response to supplementation. Twelve SNPs of the VD system genes CYP2R1 (rs10741657), CYP27B1 (rs10877012), DBP (rs4588, rs7041), VDR (rs7975232, rs731236, rs2228570, rs1544410), CYP24A1 (rs2582426, rs927650, rs2296241, rs2248137) were analyzed in a case-control design. These SNPs were correlated with $25(OH)D_3$, $1,25(OH)_2D_3$, parathyroid hormone (PTH), C-Peptide, and HbA1c concentrations in an interventional trial where patients with T2D had been supplemented with VD_3.

Patients and Methods

SNPs of the VD system: T2D susceptibility and the VD status

A case-control cohort study was conducted to investigate an association of VD system SNPs with T2D. Data from up to 553 T2D patients and 916 healthy controls were available, but sufficient DNA for genotype analysis only in 464 patients (209 women and 255 men) and 292 (138 women and 154 men) controls.

Patients were recruited from the Endocrine & Diabetes Clinic, healthy controls from the Occupational Health service of the University Hospital in Frankfurt/Main and the Blood Donor Service. $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations were available for 62 (31 women and 31 men) patients and 73 (38 women and 35 men) healthy controls.

VD intervention study

This preliminary pharmacogenetic analysis was conducted on samples from a recently published randomized trial [8], which investigated the effects of VD_3 treatment in T2D. Sixty-seven patients had been recruited (15 women, 18 men in therapy group and 16 women, 18 men in placebo group) to receive either Vigantol (VD_3, 20 drops/week, 1904 IU/d) or placebo oil for 6 months and were followed for 6 months.

Clinical parameters

Parameters were analyzed initially and after every three months until the trial's observational end at 12 months. $25(OH)D_3$ (ng/ml) and $1,25(OH)_2D_3$ (pg/ml) concentrations were measured by radioimmunoassay (RIA), PTH (pg/ml) and C-Peptide (ng/ml) by solid phase chemiluminescence assay (CLIA), and HbA1c (mmol/mol) by spectrophotometric method.

Vitamin D system genes and SNPs

Twelve SNPs in five genes were investigated: CYP2R1 (rs10741657), CYP27B1 (rs10877012), DBP (rs4588, rs7041), VDR (rs7975232, rs731236, rs2228570, rs1544410), and CYP24A1 (rs285426, rs927659, rs2296241, rs2248137).

Genomic DNA was extracted from whole blood by salting out [9]. Restriction fragment length polymorphism (RFLP) and real-time polymerase chain reaction (rtPCR) were used for genotyping. Restriction enzymes were used according to the manufacturer's instructions (New England Biolabs, Frankfurt/Main, Germany). Digestions products were separated on 3 % agarose gel and visualized by ethidium bromide staining. RtPCR analysis was conducted in Taqman (ABI7300 system) under manufacturer's conditions (Applied Biosystems, Darmstadt, Germany). To confirm accuracy, random samples of all SNPs were genotyped twice with a concordance of 100 %.

Statistical analysis

All statistical analyses were performed using Bias for Windows 10.01. Non parametric testing was chosen for the metabolic parameters due to a non-Gaussian distribution ($p \leq 10^{-4}$ in Shapiro–Wilk-test). Statistical significance was defined as $p \leq 0.05$.

SNPs within the VD system genes and VD status

Kruskal–Wallis-test was applied for the genetic effects on $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations. For each SNP a global test, comparing patients and controls, was conducted first. In case of a significant result each genotype of this SNP was compared separately. Additionally the tests for a higher risk of VD insufficiency and the SNPs were performed by Chi^2-test comparing the frequency of VD insufficient individuals between patients an healthy controls.

Bonferroni correction considered the number of SNPs in this gene (CYP2R1: 1, CYP27B1: 1, DBP: 2, VDR: 4, CYP24A1: 4), genotypes within one gene (3) and the amount of analyzed parameters (2).

VD system SNPs and T2D susceptibility

Tests for the impact of VD insufficiency on T2D risk was performed by Chi^2-test comparing the frequency of VD insufficient and VD sufficient individuals in our cohort study according to the status of disease. T2D susceptibility was investigated using Chi^2-test comparing the SNP distribution between patients and healthy controls. To allow multiple testing, all p-values were Bonferroni corrected (p_c) considering the number of genotypes (3) or alleles (2) and the amount of analyzed genes (12).

VD intervention trial

Changes in $25(OH)D_3$, $1,25(OH)_2D_3$, PTH, C-Peptide, and HbA1c concentrations during VD_3 supplementation were examined by Kruskal–Wallis-test comparing therapy and placebo group for each

genotype and study visit. For analysis of intervention associated changes of metabolic parameters within one genotype Friedmann-test was used. Bonferroni-correction was performed considering the parameters (5), genotypes (3) and number of study visits during intervention/ follow-up (4).

Results

VD and T2D risk

Patients showed overall lower $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations compared to healthy controls [$25(OH)D_3$: 18.00 vs. 12.25 ng/ml, $p = 7 \times 10^{-4}$; $1,25(OH)_2D_3$: 51.00 vs. 44.95 pg/ml $p = 0.001$]. $25(OH)D_3$ concentration is the standard parameter representing the individual VD status [10]. In our cohort study, VD insufficiency [$25(OH)D_3 < 20$ ng/ml] increases the risk of T2D by odds ratio (OR) 13.9 (confidence interval (CI) 4.8–39.2, $p < 0.001$).

VD system genes predispose to T2D

All genotyping data were in Hardy–Weinberg Equilibrium ($p > 0.05$) for each SNP. VDR rs7975232 allele "G" [40.6 % vs. 47.0 %; OR: 1.30, CI: 1.05–1.60, $p_c = 0.034$] was more frequent in T2D and there was a trend for VDR rs1544410 allele "G" (52.6 % vs. 57.9 %; OR: 1.24, CI: 1.01–1.53, $p_c = 0.098$). These results were validated testing all samples available for that gene (VDR rs7975232 "G": 43.2 % vs. 48.0 %; OR: 1.21, 95 % CI: 1.04–1.41, $p_c = 0.031$, and VDR rs1544410 "G": 53.9 % vs. 58.7 %; OR: 1.12, CI: 1.04–1.41, $p_c = 0.027$). Furthermore, the "A" allele of CYP2R1 rs10741657 (36.3 % vs. 42.1 %; OR: 1.28, CI: 1.07–1.53, $p_c = 0.016$) was more frequent among patients (▶ **Table 1**).

VD system genes affect the VD metabolism in patients with T2D

VD status and SNPs

VD insufficiency was observed in 101 and VD sufficiency in 34 individuals. None of the analyzed SNPs showed a significant association to the individual's VD status (▶ **Table 1**).

VD status and T2D associated SNPs

The VDR rs7975232 "G", VDR rs1544410 "G", and CYP2R1 rs10741657 "A" were associated with a higher T2D risk. The SNP dependent risk for T2D was analyzed in relation to VD insufficiency. This analysis did not reveal any significant impact of the three SNPs on T2D risk in this subgroup (Supplement ▶ **Table 1S**).

Vitamin D level and T2D

Analyses of VD status allow a risk-calculation but to quantify the difference of VD levels between T2D and controls a testing based on VD concentrations is necessary. To screen for SNPs that are specifically associated with lower VD concentrations in T2D patients a lower p-value was applied ($p < 0.01$). That way the specificity is raised and the per se lower VD concentrations in patients compared to controls are taken into account.

Lower $25(OH)D_3$ concentrations were detected for the genotypes CYP27B1 rs10877012 "CC" ($p_c = 4 \times 10^{-5}$), DBP rs7041 "GG" ($p_c = 0.0003$), rs4588 "CC" ($p_c = 3 \times 10^{-4}$), CYP24A1 rs2585426 "CG" ($p_c = 0.006$), and rs2248137 "CG" ($p_c = 0.001$). Additionally, the DBP genotype rs4588 "CC" showed lower $1,25(OH)_2D_3$ concentrations ($p_c = 0.005$) (▶ **Table 2**).

VD system genes affect the response to Vitamin D_3 supplementation

Sixty-five participants in the interventional study were genotyped and – with an exploratory intention – analyzed for changes in $25(OH)D_3$, $1,25(OH)_2D_3$, PTH, C-Peptide, and HbA1c. The following genotypes showed continuously higher $25(OH)D_3$ concentrations till 6 months' follow-up compared to placebo (significant/ trend): CYP27B1 rs10877012 "AC" (18.80 vs. 13.85, $p_c = 0.059$), DBP rs4588 "CC" (18.80 vs. 10.50, $p_c = 0.086$), VDR rs7975232 "AG" (20.00 vs. 10.90, $p_c = 0.034$) and VDR rs1544410 "GG" (21.10 vs. 9.90, $p_c = 0.013$) whereas the genotype CYP24A1 rs2296241 "GG" did not show any significant difference for the response to VD_3 supplementation any time (Supplement ▶ **Table 2S**).

PTH was significantly suppressed during intervention in carriers of the genotypes CYP2R1 "AG" (median difference (MD) 12.0, CI 5.0–21.0, $p_c = 0.002$), DBP rs4588 "CC" (MD 14.5, CI 8.0–24.0, $p_c < 0.001$), VDR rs2228570 "TC" (MD 13.5, CI 7.0–20.5, $p_c < 0.001$), CYP24A1 rs927650 "TT" (MD 13.6, CI 5.0–23.0, $p_c = 0.045$), CYP24A1 rs2296241 "AG" (MD13.03, CI 7.00–20.50, $p_c = 0.005$) and CYP24A1 rs2248137 "CC" (MD 14.5, CI 6.3–25.0, $p_c = 0.021$).

For changes in $1,25(OH)_2D_3$, C-Peptide or HbA_{1c} there was no significant association to any investigated SNP (data not shown).

Discussion and Conclusions

In our cohort study, we find a higher risk for T2D conferred by CYP2R1 rs10741657 "A", VDR rs7975232 "G", and VDR rs1544410 "G". These two loci control VD synthesis (CYP2R1) and VD action (VDR). A recently published GWAS identified 143 risk variants for T2D in Europeans but none of the VD pathway [11] and a study from Norway did not find any association of CYP2R1 SNPs with T2D [12]. However a recently published Mendelian randomization study on more than 890 000 individuals including the CYP2R1 SNP showed that genetically predicted higher $25(OH)D_3$ levels conferred significant protection from T2D [13]. Since genetic associations do not explain a cause-effect relation, the functional explanation for the observed effects might be due to linkage of the analyzed SNPs with other causal genes. The detection of such genes would guide to pathways of interest.

For $25(OH)D_3$ levels associations with CYP2R1 genotypes were established by GWAS and large scale population studies [14,15] while there was no effect on VD concentrations in our small amount of patients with T2D. The CYP2R1 gene codes for the key enzyme in the vitamin D metabolism for the 25-hydroxylation. How a variant of this gene, which is located near the 3′UTR affects a different function remains unclear. Potential explanations include changes in enzyme activity resulting in lower $25(OH)D_3$ synthesis, altered transcription rate, mRNA stability, substrate affinity and protein instability [16]. Linkage disequilibrium and more complex gene-gene or gene-environment interactions may affect the gene´s regulation and warrant further investigations.

For the three intronic SNPs of the VDR genes, rs1544410, rs7975232, and rs731236 (also known as BsmI, ApaI, and TaqI, respectively), associations with the VD status and also with T2D risk

▶ **Table 1** SNPs in VD system genes and susceptibility to T2D and VD status [25(OH)D_3 < 20 ng/ml].

									VD Status (25(OH)D_3)			
Gene	**SNP**	**Allele**	**Control**	**n**	**Patients**	**n**	**OR (95% CI)**	**p_c**	**<20 ng/ml n = 101**	**≥20 ng/ml n = 34**	**OR (95% CI)**	**p_c**
CYP2R1	rs10741657	A	350 (36.3%)	482	455 (42.1%)	540	1.28 [1.07–1.53]	**0.016**	70	29	0.72 [0.41–1.25]	0.299
		G	614 (63.7%)		625 (57.9%)		0.78 [0.66–0.94]		132	39	1.4 [0.8–2.46]	
CYP27B1	rs10877012	A	303 (34.9%)	434	344 (31.5%)	546	0.86 [0.71–1.04]	0.245	70	21	1.19 [0.66–2.14]	0.674
		C	565 (65.1%)		748 (68.5%)		1.17 [0.97–1.41]		132	47	0.84 [0.47–1.52]	
DBP	rs4588	A	223 (28.6%)	390	300 (27.2%)	551	0.93 [0.76–1.15]	1.000	60	18	1.17 [0.63–2.18]	0.723
		C	557 (71.4%)		802 (72.8%)		1.07 [0.87–1.31]		142	50	0.85 [0.46–1.58]	
DBP	rs7041	T	353 (46.2%)	382	489 (44.7%)	547	1.06 [0.88–1.28]	1.000	96	23	1.77 [1.0–3.14]	0.068
		G	411 (54.8%)		605 (55.3%)		1.06 [0.88–1.28]		106	45	0.56 [0.32–1-0]	
VDR	rs7975232	G	746 (43.2%)	863	518 (48.0%)	540	1.21 [1.04–1.41]	**0.031**	98	27	1.43 [0.82–2.5]	0.263
		A	980 (56.8%)		562 (52.0%)		0.83 [0.71–0.96]		104	41	0.7 [0.4–1.22]	
VDR	rs731236	T	682 (39.2%)	871	418 (39.4%)	530	1.01 [0.87–1.18]	1.000	74	27	0.88 [0.5–1.54]	0.758
		C	1060 (60.8%)		642 (60.6%)		0.99 [0.85–1.16]		128	41	1.14 [0.65–2.0]	
VDR	rs2228570	T	652 (38.5%)	847	412 (38.1%)	540	0.99 [0.84–1.15]	1.000	77	31	0.74 [0.42–1.28]	0.345
		C	1042 (61.5%)		668 (61.9%)		1.01 [0.87–1.19]		125	37	1.36 [0.78–2.37]	
VDR	rs1544410	A	844 (46.1%)	916	457 (41.3%)	553	0.82 [0.7 1–0.96]	**0.027**	76	28	0.86 [0.49–1.51]	0.700
		G	988 (53.9%)		649 (58.7%)		1.12 [1.04–1.41]		126	40	1.16 [0.66–2.03]	
CYP24A1	rs2585426	C	957 (74.6%)	641	759 (73.1%)	519	0.92 [0.77–1.11]	0.864	125	45	1.12 [0.62–2.03]	0.82
		G	325 (25.4%)		279 (26.9%)		1.08 [0.90–1.30]		57	23	0.89 [0.49–1.61]	
CYP24A1	rs927650	C	736 (53.8%)	684	607 (55.4%)	548	1.07 [0.91–1.25]	0.915	96	37	0.76 [0.44–1.32]	0.400
		T	632 (46.2%)		489 (44.6%)		0.94 [0.80–1.10]		106	31	1.32 [0.76–2.29]	
CYP24A1	rs2296241	A	366 (53.5%)	342	544 (50.7%)	537	0.89 [0.74–1.08]	0.526	108	37	0.96 [0.55–1.67]	0.996
		G	318 (46.5%)		530 (49.3%)		1.12 [0.93–1.36]		94	31	1.04 [0.60–1.80]	
CYP24A1	r2248137	G	260 (38.3%)	339	416 (36.6%)	568	0.93 [0.76–1.13]	0.985	120	43	0.85 [0.48–1.50]	0.678
		C	418 (61.7%)		720 (63.4%)		1.08 [0.88–1.31]		82	25	0.18 [0.67–2.07]	

Significant results are highlighted in bold letters. p_c: p corrected for multiple testing.

▶ **Table 2** SNPs in VD system genes and the vitamin D metabolites: $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations.

			$25(OH)D_3$ (ng/ml)			$1,25(OH)_2D_3$ (pg/ml)						$25(OH)D_3$ (ng/ml)			$1,25(OH)_2D_3$ (pg/ml)		
SNP	**Group**	**n**	**Median**	p_{global}	p_c	**Median**	p_{global}	p_c	**SNP**	**Group**	**n**	**Median**	p_{global}	p_c	**Median**	p_{global}	p_c
CYP2R1 rs10741657									VDR rs2228570								
AA	T2D	7	8.50	0.003	0.056	43.30	0.045	0.232	TT	T2D	5	7.90	0.003	1.000	47.10	0.039	1.000
	Co	13	21.20			51.00				Co	16	14.40			47.50		
AG	T2D	32	13.30		0.097	45.95		0.044	TC	T2D	28	12.65		0.0945	43.45		0.079
	Co	27	18.90			58.00				Co	38	18.65			54.50		
GG	T2D	23	10.90		0.011	44.50		0.396	CC	T2D	29	12.20		0.056	45.10		0.500
	Co	33	15.80			48.00				Co	19	28.90			54.00		
CYP27B1 rs10877012									VDR rs1544410								
AA	T2D	8	16.70	<0.001	1.000	46.05	0.058		AA	T2D	9	13.60	0.002	0.153	45.10	0.034	1.000
	Co	11	12.50			51.00				Co	10	22.50			57.50		
AC	T2D	25	12.60		0.222	44.90			AG	T2D	33	12.30		0.014	46.80		0.534
	Co	28	15.20			53.50				Co	33	20.20			55.00		
CC	T2D	29	11.00		<0.001	44.50			GG	T2D	20	12.05		1.000	43.15		0.428
	Co	34	20.45			52.00				Co	30	16.20			49.50		
DBP rs4588									CYP24A1 rs2585426								
AA	T2D	3	12.60	<0.001	1.000	30.70	0.006	1.000	CC	T2D	32	12.05	<0.001	0.086	44.75	0.031	0.075
	Co	9	15.80			38.00				Co	41	17.90			51.00		
AC	T2D	25	13.60		0.866	46.80		0.210	GC	T2D	27	13.60		0.006	44.90		1.000
	Co	29	15.60			58.00				Co	23	20.90			50.00		
CC	T2D	34	11.45		<0.001	43.45		0.005	GG	T2D	3	7.80		0.641	69.00		1.000
	Co	35	19.20			51.00				Co	9	11.40			59.00		
DBP rs7041									CYP24A1 rs927650								
TT	T2D	11	12.60	<0.001	1.000	46.80	0.014	1.000	CC	T2D	20	11.05	0.004	0.133	42.70	0.013	1.000
	Co	15	15.80			45.00				Co	20	16.20			47.50		
TG	T2D	31	13.60		0.175	45.00		0.284	TC	T2D	29	13.00		0.230	46.90		1.000
	Co	36	17.30			49.50				Co	28	17.65			51.00		
GG	T2D	20	10.95		<0.001	43.90		0.015	TT	T2D	13	11.90		0.505	40.00		0.030
	Co	22	21.20			55.50				Co	25	18.90			56.00		
VDR rs7975232									CYP24A1 rs2296241								
GG	T2D	13	11.90	0.001	1.000	39.70	0.036	0.701	AA	T2D	19	11.20	0.006	0.214	40.50	0.040	0.110
	Co	16	15.70			48.00				Co	23	14.10			58.00		
AG	T2D	31	12.30		0.046	44.50		0.117	GA	T2D	29	12.70		0.041	44.90		1.000
	Co	36	18.90			55.50				Co	32	18.05			49.00		
AA	T2D	18	13.15		0.064	46.70		1.000	GG	T2D	14	11.80		1.000	45.70		1.000
	Co	21	19.20			51.00				Co	18	19.05			51.50		

Table 2 Continued.

SNP	Group	n	25(OH)D_3 (ng/ml) Median	25(OH)D_3 (ng/ml) p_{global}	25(OH)D_3 (ng/ml) p_c	1,25(OH)$_2D_3$ (pg/ml) Median	1,25(OH)$_2D_3$ (pg/ml) p_{global}	1,25(OH)$_2D_3$ (pg/ml) p_c	SNP	Group	n	25(OH)D_3 (ng/ml) Median	25(OH)D_3 (ng/ml) p_{global}	25(OH)D_3 (ng/ml) p_c	1,25(OH)$_2D_3$ (pg/ml) Median	1,25(OH)$_2D_3$ (pg/ml) p_{global}	1,25(OH)$_2D_3$ (pg/ml) p_c
VDR rs731236									CYP24A1 rs2248137								
TT	T2D	21	11.90	0.002	0.999	41.80	0.017	0.123	GG	T2D	10	9.10	<0.001	0.919	47.50	0.048	1.000
	Co	30	16.20			50.50				Co	14	13.10			54.50		
TC	T2D	32	12.45		0.011	44.35		0.944	GC	T2D	30	12.10		0.001	44.95		0.304
	Co	35	20.20			48.00				Co	29	20.20			51.00		
CC	T2D	9	12.60		0.289	46.80		1.000	CC	T2D	22	13.50		1.000	43.90		0.066
	Co	8	22.05			60.50				Co	30	17.95			51.50		

Significant results are highlighted in bold letters. Significance: $p_{global} < 0.05$, $p_c < 0.01$. p_c: p corrected for multiple testing; T2D: Diabetes mellitus type 2; Co: Control.

have been described [17– 20] whereas other studies did not find this [21–25]. The prevalence of T2D was found higher for carriers of the rs1544410 "A" allele in an Indian [19] and German cohorts [17] but for the "G" allele in East Asians [20]. The heterogeneity of previous study results indicates a high variability of the genetic impact. Our study results present VDR 7975232 "G" VDR rs1544410 "G" as a risk factor for T2D but none of them was associated with significant changes neither of VD status nor VD concentrations.

In contrast, we found lower 25(OH)D_3 concentrations associated with the genotypes CYP27B1 rs10877012 "CC", DBP rs4588 "CC", DBP rs7041 "GG", CYP24A1 rs2585426 "CG" and CYP24A1 rs2248137 "CG" in patients. This confirms previous findings for CYP27B1 [26–29]. Since the analyzed SNP is in the promotor region of the CYP27B1 gene lower mRNA concentrations may explain the associations as this has been reported for the genotype "CC" in patients with type 1 diabetes mellitus [30] also leading to lower protein and enzyme activity. For DBP rs4588 "A" allele and "AA" genotype and rs7041 "T" allele and genotype "TT" lower VD concentrations have been reported [7,31–36]. The rs4588 C to A mutation corresponds with a deprivation of the *O*-glycosylation side of threonine [37] it can be hypothesized that hyperglycemia changes *O*-glycosydic modifications which might lower VD concentrations in T2D due to alterations in VD binding affinity [38,39]. Hereby VD supplementation improves VD concentrations in patients particularly with the rs4588 "CC" genotype, indicating a better VD binding capacity in case of high substrate availability. Also in healthy subjects the "CC" genotype is associated with higher 25(OH)D_3 level in response to VD supplementation [40]. Other genotypes have been found such as the VDR rs1544410 "GG" to be better responders to VD supplementation. Serrano et al. reported the same effect in healthy individuals after VD supplementation with retinol fortified soybean for two month [41].

Also SNPs of the CYP24A1 gene, coding for the VD degrading 24-hydroxylase [42] are associated with lower 25(OH)D_3 concentrations. We find lower 25(OH)D_3 concentrations for the genotypes rs2585426 "CG" and rs2248137 "CG". Since the genotype rs2585426 "GG" showed a trend for lower 25(OH)D_3 concentrations ($p_c = 0.087$) the "G" allele can be assumed to mediate this effect presumably via degradation.

None of the analyzed SNPs showed an association with the VD status. Combining the SNP analysis with the VD status also did not detect a significant T2D risk. In our cohort study the potential cause-effect relation leading to associations cannot be clarified. It is possible, that the impact of SNPs and VD insufficiency on T2D risk is independent of each other and not directly linked to the genetic loci that we investigated.

The odds ratio in our cohort study reveals that VD insufficiency has a modest impact on T2D risk and the impact of the SNPs is also relatively small. In conclusion the limited sample size for the VD-SNP analysis cannot detect a genetic impact of the VD status in relation to T2D risk. Therefore our results neither prove nor exclude a functional role of VD in T2D risk.

Nimitphong et al. analyzed the effect of DBP SNPs rs4588 on D_3 or D_2 supplementation in healthy subjects and showed a higher increase for the "CC" genotype compared to "AA" and "CA" which is congruent with our findings [40]. However, this effect was limited to D_3 supplementation. Two further studies confirm these results,

but showed a higher relative increase for the genotype DBP rs4588 "AA" [43,44]. One supplementation trial in T2D including VDR SNPs detected a low response for the VDR "TT" genotype [25]. This effect was not confirmed in our study which might be due to the limited sample size. Moreover only a modest dose for VD supplementation was used and possible confounding variables like age, biophysical activity, diet, and sun exposure were not addressed.

Still, our results provide preliminary evidence for a genetic control of the response to VD supplementation resulting in variable suppression of PTH in patients with T2D. Until today there is only limited knowledge about the role of VD metabolism genes on the response to VD supplementation in general and in patients with T2D in particular. The PTH plateau threshold for rising $25(OH)D_3$ levels appears to be fixed and to differ between white and black women [45] and from Chinese [46] implying a genetic mechanism in the parathyroid response to vitamin D.

Recently, a trial from Saudi Arabia recruited 204 T2D subjects for an intervention using 2000 IU/d cholecalciferol and showed significant improvements of several metabolic parameters of diabetes and lipids that also were related to genotypic variation of the VDR [47]. These findings imply, that in order to achieve optimal cardiometabolic effects any vitamin D supplementation may need to be dosed individually. Such VD effects on the glucometabolism depend on interaction with VDR both in peripheral tissues but also in the central nervous system where receptors and the activating D_3-1α-hydroxylase are expressed [48]. Furthermore VD action on the hypothalamus and the arcuate nucleus appears to regulate glucose homeostasis and body weight in animals [49].

Taken together the steroidal hormone vitamin D needs to be further characterized as an adjunct in diabetes treatment. Therefore, additional studies with higher VD doses for supplementation and larger cohorts are desirable.

Our study confirms that vitamin D deficiency is highly prevalent in type 2 diabetes and most patients are also functionally affected by low levels of the active metabolite $1,25(OH)_2D_3$. Furthermore vitamin D system genes affect the risk of type 2 diabetes and $25(OH)D_3$ concentration. But the cause-effect association remains not clarified. The response to VD_3 supplementation is influenced by genotypes regulating their magnitude and persistence of a sufficient vitamin D status and the parathyroid response. In order to confirm these preliminary results follow-up trials are necessary as well as functional studies to identify mechanisms how the VD system affects T2D pathophysiology.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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