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The role of cubilin gene polymorphisms and their influence on 25(OH)D_3 and 1,25(OH)$_2D_3$ plasma levels in type 1 diabetes patients☆

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ABSTRACT

Background: Megalin and cubilin bind and internalize the 25(OH)D_3–DBP complex in the kidney. Once the complex is internalized, 25(OH)D_3 is released and activated to 1,25(OH)$_2D_3$ the ligand for the vitamin D receptor (VDR). Supporting the important role of cubilin in this process recent findings showed that cubilin deficiency results in decrease of 25(OH)D_3 and 1,25(OH)$_2D_3$ plasma levels.
Methods: Two hundred T1D patients and healthy controls ($n = 200$) were genotyped for five polymorphisms (rs3740168, rs3740165, rs1801233, rs1801229 and rs2796835) within the cubilin gene. The polymorphisms were analyzed by RFLP or real time PCR. Statistic analyses were performed by using allele-wise and genotype-wise χ^2 testing by using BiAS software. A p-value < 0.05 was considered as significant.
Results: We found that the genotype "AA" of the rs3740165 was more frequent in T1D patients compared to healthy controls (26.7% vs. 5.1%, $p = 4 \times 10^{-7}$). Nevertheless no association between the rs3740165 polymorphism and the 25(OH)D_3 or 1,25(OH)$_2D_3$ plasma levels was found. No association with the other studied polymorphisms was observed.
Conclusion: Thus our findings reveal a novel association of the cubilin rs3740165 polymorphism with type 1 diabetes. Nevertheless how exactly this polymorphism could increase the risk to develop type 1 diabetes is subject for further investigations.

1. Introduction

The vitamin D system has been implicated in type 1 diabetes (T1D) by epidemiological, immune interventional and genetic studies. Thus polymorphisms of the vitamin D binding protein (DBP), CYP27B1 and CYP2R1 genes have been associated with this disease [1]. Furthermore in animal models the supplementation with the most active vitamin D metabolite, 1,25(OH)$_2D_3$, reduced the risk of developing T1D [2].

The synthesis of the 1,25(OH)$_2D_3$ requires two hydroxylations, one at the 25 and one at the 1-alpha positions. In the liver the 25-vitamin D hydroxylase (CYP2R1) catalyzes vitamin D_3 to 25-hydroxyvitamin D_3 (25(OH)D_3), the main circulating vitamin D metabolite. After its hydroxylation 25(OH)D_3 binds to the DBP and is delivered to the periphery. In the kidney megalin and cubilin bind and internalize the 25(OH)D_3–DBP complex, after which the 25(OH)D_3 is released and activated to 1,25(OH)$_2D_3$ by the CYP27B1 hydroxylase [3].

Cubilin is a 460-kDa peripheral membrane protein which is expressed in various tissues including renal proximal tubules, placenta, intestinal epithelium and possibly thymus among others. Recently it was demonstrated that in the kidney where the majority of systemic 1,25(OH)$_2D_3$ is produced, endocytosis of the complex 25(OH)D_3–DBP is absolutely dependent on the membrane proteins megalin and cubilin [4–6].

Moreover new findings underline the role of cubilin in the endocytic pathway of the 25(OH)D_3–DBP complex. Recent studies demonstrated that the loss of a functional cubilin in patients and in dogs result in urinary loss of the 25(OH)D_3 and subsequent decrease in 25(OH)D_3 and 1,25(OH)$_2D_3$ plasma levels [7].

Since in humans, low serum levels of 25(OH)D_3 and 1,25(OH)$_2D_3$ have been reported at the diagnosis of T1D [8] and given the importance of cubilin in the renal cell uptake of 25(OH)D_3–DBP complex, we investigated the role of polymorphisms within the cubilin gene on chromosome 10p12.33-p13 in T1D and their possible influence on the 25(OH)D_3 and 1,25(OH)$_2D_3$ plasma levels.

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2. Subjects and methods

2.1. Subjects

Type 1 diabetes was diagnosed according to the World Health Organisation criteria. All T1D patients were recruited from the endocrine outpatient clinics at the University Hospital Frankfurt am Main, Germany. The female:male ratio of the affected siblings was 1:1.03 and the median age at diagnosis was 11.5 years.

Healthy controls were volunteer blood donors from the Red Cross Blood Transfusion Center in Frankfurt am Main Germany, staff personnel or medical students from the University Hospital Frankfurt am Main Germany without a family story of autoimmune disease.

All participants were of German origin and inhabitants of the area surrounding Frankfurt am Main, Germany. The study protocol was approved by the Ethics Committee of the University Hospital Frankfurt am Main and written informed consent was obtained from all participants.

2.2. Genotyping

So far, no data correlating cubilin polymorphisms and T1D have been reported. We analyzed the role of five polymorphisms, rs3740168, rs3740165, rs1801229, rs2796835 and rs1801233 [Genbank NT_077569/AF034611 (rs1801233) according to the NCBI database] within the cubilin gene in T1D patients ($n = 200$) and healthy controls ($n = 200$). Polymorphisms were studied by restriction fragment length polymorphism (RFLP) or using real time PCR (rs2796835) with the primers or assay listed in Table 1.

2.3. Measurement of 25(OH)D_3 and 1,25(OH)$_2D_3$

25(OH)D_3 and 1,25(OH)$_2D_3$ were measured by radioimmunoassay (I125 Radioimmunoassay IA Kit; DiaSorin, Stillwater, MN). Plasma concentration of <20 ng/mL 25(OH)D_3 was defined as vitamin D insufficiency, while a range of 19.9–67 pg/ml of 1,25(OH)$_2D_3$ was considered normal according to the range of the manufacturer's instructions.

2.4. Statistical analysis

Patients and controls were compared using allele-wise and genotype-wise χ^2 testing. A $p < 0.05$ was considered as significant. Because the levels of 25(OH)D_3 and 1,25(OH)$_2D_3$ were not normally distributed, their correlation with the rsrs3740165 polymorphism were estimated by the Kruskal–Wallis test. All calculations were performed using Bias Statistical package 7.01.

3. Results

No deviations from the Hardy-Weinberg equilibrium were observed. The rs3740168, rs1801233, rs2796835 and rs1801229 polymorphisms were not found in all three genotype variants in both groups (Table 1). The rs3740165 polymorphism was prevalent in all three genotype variants and therefore we concentrated our analysis on this polymorphism.

We found a significant difference in the distribution of the rs3740165 polymorphism between T1D patients and healthy controls. Thus the genotype "AA" of the rs3740165 was more frequent in T1D patients compared to healthy controls (26.7% vs. 5.1%, $p = 4 \times 10^{-7}$).

Although not statistically significant, the analysis of the rs3740165 distribution in type 1 diabetes patients stratified according to the 25(OH)D_3 and 1,25(OH)$_2D_3$ levels revealed that 25(OH)D_3 insufficiency was more frequent in patients carrying the genotype AA in comparison to those with the genotype GG or GA (58%, 28% and 50%, respectively), while a 1,25(OH)$_2D_3$ D_3 insufficiency was found in 6.9%, 12.5% and 15.1% of the patients with the genotype AA, GG and GA, respectively.

Table 1
Distribution of the cubilin polymorphisms and their primers' sequences.

Polymorphisms	Group	Genotype (%)			*P*	Primers' sequences	Enzym
		AA	AG	GG			
rs3740165	T1D	26.7	42.7	30.5	4×10^{-7}	5′-ATT CAC TAT GGC AGT TTC-′3	Nde I
	HC	5.1	50.3	44.6		5′-CTC CTT TAC ATT GAG AGC-′3	
Polymorphisms	Group	Genotype (%)			*P*	Primers' sequences	Enzym
		GG	GC	CC			
rs2796835	T1D	0	0	100	ns†	C_15808583#	
	HC	0	0	100			
Polymorphisms	Group	Genotype (%)			*P*	Primers' sequences	Enzym
		AA	GA	GG			
rs1801233	T1D	5.8	94.2	0	ns†	5′-TAC TGA AAT TGC CTT TGT CCA-′3	Bsm I
	HC	3	97	0		5′-GAC CCA CTT AGA ATT GCT CTT TAA T-′3	
Polymorphisms	Group	Genotype (%)			*P*	Primers' sequences	Enzym
		CC	GC	GG			
rs3740168	T1D	0	3.2	96.8	ns†	5′-GCA AGA GAG CAA GCT CCT GTC TCA AAA-′3	BstBI
	HC	0	1	99		5′-CTT ATG GGT AAA AGG CAT GCA AAT TAT GGA-′3	
Polymorphisms	Group	Genotype (%)			*P*	Primers' sequences	Enzym
		GG	GA	AA			
rs1801229	T1D	4.6	3.7	91.7	ns†	5′-TTT TAG GTA AAA AAT AAA TGT CCT TA-′3	Tfi I
	HC	2.2	0	97.8		5′-GGC ATC TGT ATT TAA AGT GTT TG-′3	

T1D, Type 1 diabetes patients; HC, healthy controls; ns†, non-significant; #, by applied biosystems.

There were also no differences in the distribution of the rs374068, rs1801233, rs1801229 and rs2796835 polymorphisms between T1D patients and healthy controls (data not shown).

4. Discussion

So far, cubilin has not been investigated in association with type 1 diabetes. Nevertheless polymorphisms within genes of the vitamin D cascade as DBP, CYP27B1 and CYP2R1 were demonstrated to be associated with type 1 diabetes and to influence $25(OH)D_3$ or their mRNA expression [9,10]. Moreover there is evidence that the Vitamin D supplementation in early life reduce the risk for type 1 diabetes [11].

In the renal endocytic pathway of the $25(OH)D_3$ megalin and cubilin play an essential role. Thus once hydoxylated the $25(OH)D_3$ is delivered bound to the DBP to the target cells. In the kidney the $25(OH)D_3$ bound to DBP complex is internalized by megalin and cubilin and hydroxylated to $1,25(OH)_2D_3$ the ligand for the vitamin D receptor (VDR) [12]. Moreover in vivo megalin deficient mice or cubilin deficient dogs display a reduction in plasma of $25(OH)D_3$ or $1,25(OH)_2D_3$. Moreover microalbuminurie in type 1 diabetes is associated with enhanced excretion of the endocytic multiligand receptors megalin and cubilin [13].Thus a dual receptor mechanism involving both cubilin and megalin is essential to maintain sufficient substrate for the synthesis of $1,25(OH)_2D_3$ [6,12].

Since diverse genes of the vitamin D cascade have been associated with T1D, in the present study, we analyzed mainly the role of the rs3740165 polymorphism within the cubilin gene in the susceptibility to type 1 diabetes in the German population. According to our data since the allele A of the rs3740165 polymorphism was more frequent in type 1 diabetes in comparison to healthy control, subsequently we postulate that the allele A could represent a risk factor for susceptibility to type 1 diabetes.

Although mutations within cubilin could result in a $1,25(OH)_2D_3$ deficiency secondary to impaired renal uptake of the $25(OH)D_3$–DBP complex [6,7], we did not observe a correlation between the rs3740165 polymorphism and the $25(OH)D_3$ or $1,25(OH)_2D_3$ plasma levels. Nevertheless we cannot discard that the rs3740165 polymorphism, which results in a coding sequence synonym change [Pro→Pro] together with regulatory polymorphisms could alter the function of cubilin. Thus the rs3740165 polymorphism within the cubilin gene cannot be excluded as a candidate gene for type 1 diabetes. Therefore these findings need to be corroborated in larger numbers.

In conclusion, besides our limited numbers and the very polymorphic gene region of the cubilin gene do not allow a final conclusion. Nevertheless this is the first report on an association of a cubilin gene variant with type 1 diabetes.

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