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**Highlights**

- 95 SNPs within the *CUBN* gene were genotyped in 716 patients with T2DM and 542 controls
- rs11254375_G/T (OR=1.482), rs7071576_A/G (OR=1.219), rs6602175_G/T (OR=0.822), rs1801224_G/T (OR=0.830), and rs4366393_A/G (OR=0.829) were linked to T2DM risk
- rs41301097 was strongly associated with higher 25(OH)D levels

**Abstract**

Accumulating evidence suggests a potential implication of vitamin D biological network in the pathogenesis of diabetes mellitus. The megalin-cubilin endocytotic system constitutes a key transport structure, with a modulating role in vitamin D metabolism. We aimed to assess the contribution of variants in the *CUBN* gene to the genetic risk of Type 2 Diabetes Mellitus (T2DM). 95 polymorphisms within *CUBN* were genotyped in 716 patients with T2DM and 542 controls of Greek origin. Samples were analyzed on Illumina Human PsychArray. Permutation test analysis was implemented to determine statistical significance. Twenty-five-hydroxy-vitamin-D [25(OH)D)] levels were measured in a sub-group of participants (n=276).
analysis associated rs11254375_G/T (pemp=0.00049, OR=1.482), rs6602175_G/T (pemp=0.016, OR=0.822), rs1801224_G/T (pemp=0.025, OR=0.830), rs4366393_A/G (pemp=0.028, OR=0.829) and rs7071576_A/G (pemp=0.04, OR=1.219) with T2DM. Mean 25(OH)D concentrations were significantly lower in patients with T2DM compared to controls (16.70 ± 6.69 ng/ml vs 18.51 ± 6.71 ng/ml, p<0.001), although both groups were vitamin D deficient. In a further quantitative analysis, rs41301097 was strongly associated with higher 25(OH)D concentrations (p=5.233e-6, beta=15.95). Our results indicate a potential role of CUBN gene in T2DM genetic susceptibility in the Greek population. These findings may also denote an indirect effect of vitamin D metabolism dysregulation on the pathogenesis of T2DM. Further studies are required to replicate our findings and clarify the complex underlying mechanisms.

**Keywords:** Type 2 Diabetes; Vitamin D; Cubilin; SNP; Gene

**Abbreviations:** T2DM: Type 2 diabetes mellitus; DPP4: Dipeptidyl peptidase-4; SGLT2: Sodium glucose co-transporter 2; GLP-1: Glucagon-like peptide-1.

### 1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a multifactorial disorder, resulting from complex interactions between genetic, environmental and lifestyle components [1]. The risk for developing T2DM is strongly inherited, with existing evidence suggesting that first degree relatives of T2DM patients, present a 3-fold greater probability of developing diabetes, compared to individuals without a positive family history [2]. Candidate gene approaches investigating T2DM genetic susceptibility, have mainly focused on genetic loci involved in the well-established pathogenetic pathways of the disease, including deficits in beta-cell function and insulin action, impaired glucose metabolism and insulin resistance [3]. However, they have - so far - produced conflicting
inconclusive results, primarily due to small sample sizes, ethnic diversity of studied populations, variation in environmental exposures, gene-gene and gene-environment interactions [4].

During the past few years and as a result of the progress in genotyping techniques, novel associations between T2DM and genetic variants have been brought into light, leading to a better understanding of the mechanisms involved in the pathophysiology of the disease. In this context, the biological network of vitamin D has been only recently linked to the pathogenesis of T2DM [5,6]. There is data pointing towards a vitamin D role in the regulation of mechanisms related to both insulin secretion and sensitivity, in a way however, that is not yet completely understood [7,8].

Megalin and cubilin (encoded by the CUBN gene in humans), are endocytic receptors highly expressed in the endocytic apparatus of the renal proximal tubule [9]. Cubilin is the physiological receptor for intrinsic factor-vitamin B₁₂ in the gut and for albumin in the kidney [10]. The megalin-cubilin endocytotic system also constitutes a key transport structure, with a modulating role in vitamin D metabolism [11]. More specifically, the circulating twenty-five-hydroxy-vitamin-D [25(OH)D] / Vitamin D Binding Protein (VDBP) complex is endocytosed into the proximal tubular cell via megalin, an apical-membrane receptor and the largest member of the Low-density Lipoprotein receptor super family [10]. Megalin-mediated endocytosis of 25(OH)D/VDBP, is assisted by the receptor-associated protein (RAP) and cubilin. The latter, is needed for sequestering VDBP on the cell surface before its internalization by megalin [12]. The aforementioned system plays a key role in the delivery of 25(OH)D to the 25-hydroxyvitamin D-1-α-hydroxylase in the kidney, where 25(OH)D molecules bound to VDBP taken up through this receptor pathway, are further transformed to calcitriol [13].
Mutations in *CUBN* gene are known to result in the Imerslund-Gräsbeck syndrome, characterized by megaloblastic anemia and proteinuria, whereas recent research has identified specific *CUBN* Single Nucleotide Polymorphisms (SNPs) as potential risk factors for neural tube defects, albuminuria and End Stage Renal Disease (ESRD) [14]. Indirect evidence of a relationship between *CUBN* and diabetes has been suggested by isolated case reports, describing the co-existence of Imerslund-Gräsbeck syndrome and Type 1 Diabetes Mellitus (T1DM) in the same individual [15,16].

Despite the progress been made in understanding the physiology of cubilin network and its critical role within vitamin D homeostasis, there is still scarce evidence connecting this system with human pathology in general, and diabetes in particular. In this study, we aimed to explore potential differences of *CUBN* variant frequency between an elderly T2DM population and non-diabetic controls and assess a potential effect of *CUBN* variants on 25(OH)D levels.

2. Methods

2.1 Study population

Subjects with an established T2DM diagnosis were consecutively recruited from the outpatient diabetes clinics of AHEPA University Hospital of Thessaloniki and General University Hospital of Alexandroupolis, Greece. Community-dwelling individuals from the same regions, in whom normal glycemia was documented, were included as controls. Each participant completed a questionnaire in order information regarding age, sex, medical and family history to be obtained by the research team.

The inclusion criteria for the control group were as follows: (a) > 65 years of age, (b) glycated hemoglobin (HbA1c) < 6.5% (48mmol/mol), (c) fasting plasma glucose (FPG) <126 mg/dl, (d) no past history of T2DM diagnosis according to the aforementioned
criteria, (e) no prior or current administration of anti-diabetic drugs and (f) no current administration of vitamin D supplements or any other medication that can affect blood glucose, such as corticosteroids and antipsychotics.

Diagnosis of T2DM was based on the criteria proposed by the American Diabetes Association [17]. People with T2DM receiving drugs that have the potential to increase blood glucose levels, such as corticosteroids and antipsychotics, or vitamin D supplements were excluded from the study. All participants were residents of northern Greece, central Macedonia and Thrace and were of Greek ethnicity and origin.

2.2 Clinical and biochemical measurements

Clinical information including family history of T2DM, age at diagnosis, ancestry and present medications were obtained from self-reported data and medical records. Anthropometric measurements were performed with the subject wearing light clothing and without shoes. For all subjects, body weight and height were measured using a scale and a wall-mounted meter. Body Mass Index (BMI) was computed as weight (in kilograms) divided by height (in meters) squared. Waist circumference (in centimeters) was measured in the middle between the 12th rib and the iliac crest.

Blood samples were drawn in the morning, after a 12-h overnight fast by antecubital venepuncture and stored at -20°C prior to analysis. Glucose measurements were performed using the Cobas INTEGRA clinical chemistry system (D-68298; Roche® Diagnostics, Mannheim, Germany). Reference ranges and inter- and intra-assay coefficients of variation (CVs) for glucose were 70 - 110 mg/dl and 0.99 and 3.5%, respectively. HbA1c was measured using the ADAMS HA-8160 high-performance liquid chromatography (HPLC) method (A. Menarini® Diagnostics, Florence, Italy).
In a subgroup of the study population (200 out of 716 patients with T2DM and 74 out of 542 controls), 25(OH)D levels were determined between November and April, in order to diminish the effects of sun exposure on vitamin D concentrations, given that this is the period of the year with the lowest exposure to Ultraviolet B radiation in the Greek region [18]. 25(OH)D concentrations were measured by competitive electrochemiluminescent immunoassay on the Roche® Modular E170 (cat. no. 05894913190; Roche® Diagnostics, Laval, Canada). This assay has a lower reporting limit of 3.8 ng/ml, and at concentrations of 24, 38 and 62 ng/ml the within-run CVs are 5.1, 3.1 and 7.1% and total CVs 12.1, 7.4 and 10.6%, respectively [19]. We measured 25(OH)D instead of the active form of vitamin D (1,25-dihydroxyvitamin D), as indicated by the Institute of Medicine recommendations for Vitamin D assessment in the clinical setting [20]. This is because 25(OH)D levels more accurately reflect vitamin D status, since they are not influenced by Parathyroid Hormone and other hormone concentrations [21].

2.3 Genotyping

This study was designed to identify a putative role of vitamin D associated variations in a T2DM Greek population in a case-control study design. Genomic DNA was extracted from peripheral blood (QIAamp DNA blood kit; QIAGEN, Hilden, Germany) in 716 patients with T2DM and 542 diabetes-free controls. Samples were genotyped on Illumina Infinium PsychArray (603132 SNPs, 559921 of those are submitted to dbSNP as rs). For our analysis we used an initial SNP call rate threshold of 0.95. For our individual quality control, we removed any individuals with a call rate less than 0.98, absolute value of inbreeding coefficients over 0.2 and with a sex phenotype that deviated from their genotypic sex. For our SNP quality control, we removed any SNP
that failed the call rate threshold of 0.98, the call rate difference threshold of 0.02 between cases and controls, and a Hardy-Weinberg Equilibrium (HWE) p-value of $10^{-6}$ for controls and $10^{-10}$ for cases. Monomorphic (invariant) markers were kept at the filtering stage, but removed during the subsequent parts of the analysis. Genetic relatedness (Identity-by-descent-IBD) and principal components (eigenvectors) were calculated completing quality control, removing any outliers and one individual out of every pair that demonstrated genetic relatedness ratio over 0.1875. Polymorphisms of *CUBN* were selected using a window of 20kb upstream and downstream. For the purpose of our analysis, PLINK software suite and EIGENSOFT suite were used [22].

### 2.4 Statistical analysis

Statistical analyses were performed using PLINK v1.9 and SPSS software. Differences between case and control groups were examined using the Student t-test or Pearson chi-square test using SPSS Inc. for Windows, Version 21.0 (IBM, Foster City, CA, USA). Data are presented as mean ± standard deviation. P-value <0.05 determined the level of significance.

The allele-specific odds ratios (ORs) for T2DM were calculated using logistic regression analysis by implementing PLINK statistical package [23,24]. The results were further evaluated for their significance, by considering multiple testing correction. Even though Bonferroni correction is the initial approach when dealing with association studies, Bonferroni leads to an inflation in false-negative results, by decreasing the nominal type 1 error rate to far below the alpha threshold [25]. SNPs in a gene region are very often highly correlated, which leads to positively correlated tests. By acknowledging this, we employed in our study two methods to assess the significance of our results. The first one, is a permutation test. It employs shuffling the sample
phenotypes and reporting the empirical p-values ($p_{\text{perm}}$) [26]. In this test, we followed the adaptive permutation procedure, as performed by PLINK [23], which gives up permuting SNPs that are projected to be clearly non-significant. The second one is the method of calculating the number of effective SNPs ($M_{\text{eff}}$) as reported by Gao et al. [27]. This method calculates the number of theoretical SNPs that capture the most variance through principal component analysis. Both of these methods have been demonstrated to be the best performing methods for multiple testing correction when dealing with gene regions [28]. For these analyses, the monomorphic SNPs were excluded.

In the subgroup of study population, where 25(OH)D levels were available, a further Wald’s test analysis was undertaken. This test can capture the variance of 25(OH)D levels in relation to the studied SNPs. Vitamin D levels and SNPs were correlated, with T2DM as a co-variance. Effective SNPs method was implemented for significance evaluation with $p=0.05/115=4.38\times10^{-4}$ as a cut-off.

2.5 Ethical considerations

All study procedures conformed to the declaration of Helsinki and its later amendments. The study protocol was approved by the Ethics Committee of the Aristotle University of Thessaloniki. Written informed consent was obtained from all participants.

3. Results

3.1 Demographic, anthropometrical and biochemical features

716 people with T2DM and 542 controls (1258 individuals in total) fulfilled the relevant criteria and were included in the analysis. Female subjects predominated in the study population in both the T2DM and the control group (52.2 and 59.9%, respectively).
respectively). Mean diabetes duration among patients was 14.39 ± 9.29 years. Patients and controls were found to significantly differ in terms of body weight (84.96 ± 16.84 vs 78.73 ± 14.11 kilograms respectively, p<0.001), BMI (31.73 ± 6.77 vs 29.79 ± 5.34 kg/m² respectively, p<0.001), waist circumference (104.62 ± 15.03 vs 102.10 ± 11.94 cm respectively, p=0.001), age (68.93 ± 9.53 vs 73.53 ± 7.15 years respectively, p<0.001), FPG (153.16 ± 53.71 vs 100.30 ± 13.53 mg/dl respectively, p<0.001) and HbA1C (7.29 ± 1.27 vs 5.32 ± 0.56 % respectively, p<0.001) values. Table 1 presents the demographical, anthropometric and biochemical features of the two groups.

3.2 Correlation between CUBN polymorphisms and T2DM

No deviations from the HWE were observed. 95 SNPs were identified, excluding monomorphic SNPs. Allele frequencies of five SNPs (rs11254375, rs7071576, rs6602175, 1801224, and rs4366393) were found to significantly differ between T2DM cases and controls (Table 2). In particular, permutation analysis yielded the following associations between genetic variants and T2DM: rs11254375_G/T: p_emp=0.00049, OR=1.482, rs6602175_G/T: p_emp=0.016, OR=0.822, rs1801224_G/T: p_emp=0.025, OR=0.830, rs4366393_A/G: p_emp=0.028, OR=0.829 and rs7071576_A/G: p_emp=0.04, OR=1.219 (Table 3). Fig. 1 showcases the linkage disequilibrium matrix of the extended haplotype and its relation to our top SNPs.

3.3 Vitamin D concentrations

Mean 25(OH)D levels were significantly lower in the subgroup of patients with T2DM in which Vitamin D was measured (n=200), compared to the subgroup of controls (n=74) (16.70 ± 6.69 ng/ml vs 18.51 ± 6.71 ng/ml respectively, p<0.001) (Table 4). However, both patients and controls were found to be vitamin D deficient, according
to the United States Endocrine Society criteria [sufficiency: serum 25(OH)D > 30 ng/ml; insufficiency 21-29 ng/ml, deficiency < 20 ng/ml] [29]. In a further quantitative analysis, rs41301097 was strongly associated with higher 25(OH)D levels (p=5.233e-6, beta=15.95). Table 5 provides details about the medications received and the renal function in this subgroup of study population.

4. Discussion

To the best of our knowledge, this is the first study investigating the association between polymorphisms in the CUBN gene and T2DM. This is also the first study to assess the frequency of CUBN variants in a population consisted entirely of Greeks. Our results indicate that CUBN variants might share a both protective and predisposing role to T2DM genetic risk and reconfirm the hypothesis that vitamin D biodynamics are implicated in the pathogenesis of diabetes mellitus. Studies assessing potential effects of vitamin D deficiency on glucose homeostasis have so far yielded conflicting results. Observational studies showed that decreased 25(OH)D concentrations are associated with increased incidence of T2DM and impaired β-cell function [5]. On the other hand, intervention randomized control trials with vitamin D supplementation failed to establish an effect on glucose regulation or T2DM incidence [7]. A meta-analysis of ten randomized control trials with vitamin D supplementation in people with prediabetes did not show any effect on insulin resistance [30]. In a similar way, recently published results proved that among individuals at high risk for developing T2DM, vitamin D supplementation did not result in a significantly lower risk of diabetes compared to placebo [31]. However, this study could be criticized for including subjects with relatively high baseline levels, thus being less likely to benefit from such an intervention.
Our results suggest that \textit{CUBN} rs11254375, rs7071576, rs6602175, rs1801224 and rs4366393 polymorphisms are associated with T2DM. These intronic polymorphisms have not been previously correlated with T2DM, whereas it is still unclear whether they lead to a modified function of the encoded protein [32]. As a result, the exact mechanistic pathways that associate these SNPs with T2DM warrant further investigation by future studies.

In a previous study, 200 patients with T1DM and 200 healthy controls were genotyped for five polymorphisms (rs3740168, rs3740165, rs1801233, rs1801229 and rs2796835) within the \textit{CUBN} gene [33]. The allele A of the rs3740165 polymorphism was more frequently detected in T1DM group compared to controls. The results of this study, when combined with our findings, might suggest a role of \textit{CUBN} polymorphisms in the development of dysglycemia, irrespectively of the type of diabetes. In contrast to our observation, the authors failed to establish a relationship between rs3740165 or other SNPs and 25(OH)D or 1,25(OH)\textsubscript{2}D concentrations.

Recent works have pointed towards a significant relationship between the \textit{CUBN} gene and the risk of diabetic microvascular and macrovascular complications. Both Genome Wide Association Studies (GWAS) and candidate gene studies have identified \textit{CUBN} as a gene locus of albuminuria, regardless of diabetes status [34]. In a GWAS discovery meta-analysis of 2,191,945 SNPs in up to 54,450 participants of 20 studies, Teumer et al. [35] reconfirmed the association between \textit{CUBN} locus and microalbuminuria in subjects with diabetes. In a recently published exome-wide association study including a large number of individuals of European ancestry, a rare missense (A1690V) variant in \textit{CUBN} was associated with albuminuria in subjects with and without diabetes [36]. Moreover, a relationship between ESRD and the \textit{CUBN} SNP rs1801239 has been demonstrated in African Americans with T2DM [37]. Enhanced cubilin urinary
excretion has been documented in patients with T1DM and microalbuminuria [38], suggesting that modified cubilin function and/or expression is involved in the pathogenesis of diabetic nephropathy (DN) [39]. Albert et al. [40], demonstrated that in a Caucasian T2DM cohort, the CUBN CC or C-risk-allele of rs1801239 was correlated with DN [OR 2.04 (1.07-3.87), p = 0.03] and peripheral artery disease [OR 2.08 (1.12-3.88), p = 0.021]. Whether the aforementioned associations are casual, remains to be clarified. However, considering the sum of these findings, a role for variants in the CUBN gene in the genetic predisposition not only of diabetes itself, but of the disease’s complications as well, could be postulated.

Although the primary purpose of this study was to assess the contribution of CUBN polymorphisms to T2DM genetic risk, our findings also confirm the existence of the previously described “Mediterranean paradox”, according to which, despite high levels of sunshine, vitamin D deficiency is highly prevalent among habitants of the Mediterranean countries [41]. The exact factors that contribute to the development of this paradox have not been fully elucidated; however, racial, social and cultural habits, as well as the absence of preventive strategies seem to mitigate the benefits of sun exposure in this population [42]. Our results are also in agreement with previous reports which demonstrated that old age is an independent risk factor for vitamin D deficiency, mainly due to limited physical activity and sun exposure [43]. Decreased cutaneous synthesis and dietary intake of vitamin D have been proposed as additional explanations for this observation [44]. In our analysis, Wald’s test significantly correlated CUBN rs41301097 with higher 25(OH)D concentrations among study participants in whom 25(OH)D was measured. This subgroup was relatively well characterized in terms of factors that influence Vitamin D biodynamics. For example, subjects had preserved renal function, therefore, the observed association between the SNP and 25(OH)D
levels was unaffected by potential confounders, including diabetic nephropathy status. Still, it should be noted that this missense polymorphism was rare in our cohort, which limits the extraction of definite conclusions.

Our study is not free of limitations. First, study participants were recruited from only two major Greek diabetes centers, a fact that restricted the number of subjects included. Second, the diagnosis of T2DM was established according to FPG and HbA1C values, since participants were not evaluated by Oral Glucose Tolerance Test (OGTT), which is the method with the greater sensitivity for the diagnosis of diabetes [17]. It is known that HbA1C and OGTT categorize different patients with diabetes, with the first identifying chronic elevations in blood glucose and the latter providing a more predictive value in post-prandial glucose levels [45]. As a result, it is possible that people with prediabetes might have been falsely included in our sample. In addition, vitamin D measurements were obtained in a small proportion of the study population, since the restriction of sampling between November and April, limited the number of available samples. Finally, the two study groups presented differences with respect to a number of baseline characteristics, including mean age and BMI of participants. The minimum 65-year age cutoff for the control group was set in order to minimize the probability that T2DM will present at a later stage in the course of life of these people. However, this strategy might have resulted in a relatively high degree of penetration of protective genetic variants in the control group. Respectively, the lower BMI and body weight values of controls, could be attributed to sarcopenia, which is often observed among the elderly [46].

In conclusion, our results indicate a potential role of CUBN gene in T2DM genetic susceptibility in a population of Greek origin. However, whether these findings are applicable to other ethnic groups is uncertain, given that striking differences in
haplotypic structure of $CUBN$ between European and African populations have been previously reported [47]. Our findings may also denote an indirect effect of vitamin D metabolism dysregulation on the pathogenesis of T2DM. Further studies are required to replicate our findings and clarify the complex underlying mechanisms.

**Declaration of interest:** Authors report no conflict of interest.

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Informed consent: Informed consent was obtained from all participants.

Ethical approval: The study protocol was approved by the Ethics Committee of the Aristotle University of Thessaloniki.

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Figure legend

Fig. 1: A linkage disequilibrium (LD) plot that showcases the LD matrix of the extended haplotype and its relation to our top single nucleotide polymorphisms
Tables

Table 1. Demographical, anthropometric and biochemical features of patients and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T2DM (n=716)</th>
<th>Controls (n=542)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.93 ± 9.53</td>
<td>73.53 ± 7.15</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>52.2% / 47.8%</td>
<td>59.9% / 40.1%</td>
<td>p=0.006</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.96 ± 16.84</td>
<td>78.73 ± 14.11</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.73 ± 6.77</td>
<td>29.79 ± 5.34</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104.62 ± 15.03</td>
<td>102.10 ± 11.94</td>
<td>p=0.001</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.29 ± 1.27</td>
<td>5.32 ± 0.56</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>153.16 ± 53.71</td>
<td>100.30 ± 13.53</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td></td>
<td></td>
<td>p=0.05</td>
</tr>
<tr>
<td>(ml/min/1.73m²)</td>
<td>79.7 ± 3.36</td>
<td>83.2 ± 5.29</td>
<td></td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>14.39 ± 9.29</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. Glomerular filtration rate was estimated using the MDRD equation. **Abbreviations:** T2DM: Type 2 diabetes mellitus; F: female; M: male; min: minutes; m: meters; cm: centimeters; kg: kilograms; BMI: Body Mass Index; HbA1C: glycated hemoglobin
Table 2. Frequencies of significant *CUBN* SNPs in T2DM and control groups

<table>
<thead>
<tr>
<th>SNP</th>
<th>Major &gt;&gt; Minor allele</th>
<th>Frequency of minor allele in T2DM patients</th>
<th>Frequency of minor allele in controls</th>
<th>Pperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11254375</td>
<td>T&gt;&gt;G</td>
<td>0.1942</td>
<td>0.1399</td>
<td>0.00049</td>
</tr>
<tr>
<td>rs6602175</td>
<td>T&gt;&gt;G</td>
<td>0.3593</td>
<td>0.4054</td>
<td>0.01697</td>
</tr>
<tr>
<td>rs4366393</td>
<td>G&gt;&gt;A</td>
<td>0.4759</td>
<td>0.5227</td>
<td>0.02841</td>
</tr>
<tr>
<td>rs1801224</td>
<td>T&gt;&gt;G</td>
<td>0.4013</td>
<td>0.4467</td>
<td>0.02462</td>
</tr>
<tr>
<td>rs7071576</td>
<td>G&gt;&gt;A</td>
<td>0.2966</td>
<td>0.257</td>
<td>0.04293</td>
</tr>
</tbody>
</table>

**Abbreviations:** *CUBN*: Cubilin; *T2DM*: Type 2 diabetes mellitus; *SNP*: Single Nucleotide Polymorphism;

Pperm: permutation analysis p-value
Table 3. Polymorphisms in *CUBN* gene significantly correlated with T2DM risk

<table>
<thead>
<tr>
<th>SNP</th>
<th>Major &gt;&gt; Minor allele</th>
<th>( p_{\text{perm}} )</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11254375</td>
<td>T&gt;&gt;G</td>
<td>0.00049</td>
<td>1.482</td>
<td>1.192</td>
<td>1.844</td>
<td>0.168</td>
</tr>
<tr>
<td>rs6602175</td>
<td>T&gt;&gt;G</td>
<td>0.01697</td>
<td>0.822</td>
<td>0.697</td>
<td>0.970</td>
<td>0.381</td>
</tr>
<tr>
<td>rs1801224</td>
<td>T&gt;&gt;G</td>
<td>0.02462</td>
<td>0.830</td>
<td>0.705</td>
<td>0.976</td>
<td>0.423</td>
</tr>
<tr>
<td>rs4366393</td>
<td>G&gt;&gt;A</td>
<td>0.02841</td>
<td>0.829</td>
<td>0.706</td>
<td>0.973</td>
<td>0.498</td>
</tr>
<tr>
<td>rs7071576</td>
<td>G&gt;&gt;A</td>
<td>0.04293</td>
<td>1.219</td>
<td>1.018</td>
<td>1.460</td>
<td>0.277</td>
</tr>
</tbody>
</table>

Abbreviations: T2DM: Type 2 diabetes mellitus; SNP: Single Nucleotide Polymorphism; \( p_{\text{perm}} \): permutation analysis p-value; OR: Odds Ratio; CI: Confidence Interval; MAF: Minor Allele Frequency
Table 4. 25(OH)D concentrations assessed in a subgroup of the total study population

<table>
<thead>
<tr>
<th>Group</th>
<th>25(OH)D concentrations (ng/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2DM</td>
<td>16.70 ± 6.69</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>18.51 ± 6.71</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>18.03 ± 6.74</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation.

25(OH)D levels were determined in 200 out of 716 patients with T2DM and in 74 out of 542 controls.

**Abbreviations:** T2DM: Type 2 diabetes mellitus; 25(OH)D: 25-hydroxy-vitamin D
Table 5. Features of patients and controls in whom vitamin D was measured

<table>
<thead>
<tr>
<th>Antidiabetic drugs</th>
<th>T2DM</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>58.1%</td>
<td>-</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>9.5%</td>
<td>-</td>
</tr>
<tr>
<td>Metformin plus DPP4 inhibitor</td>
<td>17.6%</td>
<td>-</td>
</tr>
<tr>
<td>GLP-1 receptor agonists</td>
<td>1.4%</td>
<td>-</td>
</tr>
<tr>
<td>SGLT2 inhibitors</td>
<td>1.4%</td>
<td>-</td>
</tr>
<tr>
<td>Insulin plus metformin</td>
<td>5.4%</td>
<td>-</td>
</tr>
<tr>
<td>Diet plus exercise</td>
<td>6.6%</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antihypertensive drugs</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>25.7%</td>
<td>32.2%</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme</td>
<td>9.5%</td>
<td>14.4%</td>
</tr>
<tr>
<td>inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>12.2%</td>
<td>13.4%</td>
</tr>
<tr>
<td>None or unknown</td>
<td>52.6%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Renal function
<table>
<thead>
<tr>
<th>Serum creatinine (mg/dl)</th>
<th>0.87 ± 0.31</th>
<th>0.87 ±0.26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular filtration rate (ml/min/1.73m²)</td>
<td>Females 71.8 ± 2.23 / Males 83.2 ± 3.44</td>
<td>Females 77.5 ± 1.48 / Males 78.4 ± 4.52</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. Glomerular filtration rate was estimated using the MDRD equation.

25(OH)D levels were determined in 200 out of 716 patients with T2DM and in 74 out of 542 controls.