Research Article

T-Cell Cytokine Gene Polymorphisms and Vitamin D Pathway Gene Polymorphisms in End-Stage Renal Disease due to Type 2 Diabetes Mellitus Nephropathy: Comparisons with Health Status and Other Main Causes of End-Stage Renal Disease

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Background. T-cell cytokine gene polymorphisms and vitamin D pathway gene polymorphisms were evaluated as possibly associated with end-stage renal disease (ESRD) resulting from type 2 diabetes mellitus (DM) nephropathy. Methods. Studies were conducted among hemodialysis (HD) patients with ESRD due to type 2 DM nephropathy, chronic glomerulonephritis, chronic infective tubulo-interstitial nephritis, and hypertensive nephropathy as well as in healthy subjects. A frequency distribution of T-cell-related interleukin (IL) genes (IL18 rs360719, IL12A rs568408, IL12B rs3212227, IL4R rs1805015, IL13 rs20541, IL28B rs8099917, IL28B, and rs12979860) and vitamin D pathway genes (GC genes: rs2298849, rs7041, and rs1155563; VDR genes: rs2228570, rs1544410; and RXRA genes: rs110776909, rs10881578, and rs749759) was compared between groups. Results. No significant differences in a frequency distribution of tested polymorphisms were shown between type 2 DM nephropathy patients and controls. A difference was found in IL18 rs360719 polymorphic distribution between the former group and chronic infective tubulo-interstitial nephritic patients (P_trend = 0.033), which also differed in this polymorphism from controls (P_trend = 0.005). Conclusion. T-cell cytokine and vitamin D pathway gene polymorphisms are not associated with ESRD due to type 2 DM nephropathy in Polish HD patients. IL18 rs360719 is probably associated with the pathogenesis of chronic infective tubulo-interstitial nephritis.

1. Introduction

Diabetes mellitus (DM) is the most common cause of end-stage renal disease (ESRD) in many hemodialysis (HD) centers. In Australia and New Zealand, the incident ESRD population (1991–2005) who began renal replacement therapy (RRT) included 30.0% type 2 DM and 4.5% type 1 DM subjects [1]. In the HEMODIALYSIS (HEMO) study, the group of HD patients comprised approximately 45% of DM subjects [2]. Diabetic ESRD patients compared to nondiabetic ESRD subjects show higher both mortality rate [3] and prevalence of coronary artery disease (CAD) [4], are more prone to severe infections [5] and worse response to hepatitis B vaccination [6], and more often suffer from adynamic bone disease associated with low serum parathyroid hormone (PTH) levels [7]. In this paper we will focus on ESRD due to type 2 DM nephropathy. Together with altered glucose metabolism and insulin resistance, deficiency of vitamin D [8] and aberrant T-cell cytokine balance [9] were found to be associated with...
Table 1: HRM and RFLP conditions for the identification of polymorphisms genotyped in the vitamin D pathway related genes.

| Gene symbol | rs number | Alleles | Primers for PCR amplification (5'-3') | Annealing temp. (°C) | PCR product length (bp) | HRM analysis Melting temp. range (°C) | Restriction enzyme | Restriction fragment length (bp) |
|-------------|-----------|---------|--------------------------------------|----------------------|-------------------------|--------------------------------------|-------------------|-------------------------------|
| GC          | rs7041    | G/T     | F:GGAGGTGAGTTATGGAAACAGC R:GCATTAGCTCAGTGATGAGTC | 66.3                 | 493                     |                                      | HaeIII            | T = 493                        |
|             | rs1155563 | C/T     | F:GGTTATCTAACGACTGCTCTTGCA R:ATGTGATCTCAGCTGACTCC | 63.0                 | 116                     | 71–78                                | RFLP analysis     | T = 493                        |
|             | rs2298849 | C/T     | F:TCCACTGGCAAAACACATTAC R:GGGACATCGATATTATTCG | 60.6                 | 118                     |                                      | Restriction enzyme | T = 493                        |
|             |           |         |                                      |                      |                         |                                      | Restriction fragment length (bp) | G = 414 + 79       |
| RXRA        | rs1081578 | A/G     | F:TCTTGAGCAATGCGAGCAAG R:CCACAGTCACACATCAATC | 60.6                 | 75                      | 80–90                                | BstXI             | A = 382 G = 243 + 139        |
|             | rs10776909| C/T     | F:CCGCTTGGCTGCTGCTCA R:ACCTCAGCGGCGCTTGGAG | 60.6                 | 95                      | 82–92                                |                  |                               |
|             | rs749759  | A/G     | F:ATAGGGCTTGGCTGCTTAGA R:CTCCAGCAGCGGCAAGTGA | 62.6                 | 382                     |                                      |                  |                               |
| VDR         | rs1544410 | A/G     | F:GGAGACACAGATAAGGAAATAC R:CCGCAAGAAACCTCAGAATAAC | 60.6                 | 248                     |                                      | FspI              | A (B) = 248 G (b) = 175 + 73 |
|             | rs2228570 | C/T     | F:GCACCTGCCTGCTGCTGAC R:ACCTCCTGTCTGCTGTCGCT | 72.5                 | 341                     |                                      | FokI              | C (F) = 341 T (f) = 282 + 59  |

*aHRM analysis: high resolution melt analysis.

bRFLP analysis: restriction fragment length polymorphism analysis.
Table 2: Characteristics of hemodialysis patients (n = 893).

| Parameter                                      | Type 2 DM nephropathy | Other causes of ESRD | P value |
|------------------------------------------------|-----------------------|----------------------|---------|
| **Demographic data**                          |                       |                      |         |
| Male sex, n (% of all)                        | 201 (54.9)            | 307 (58.3)           | 0.337b  |
| Age at RRT beginning, years                  | 62.9 ± 14.1           | 57.2 ± 17.2          | <0.0001c|
| RRT duration, years                          | 3.29 (0.06–28.0)      | 4.42 (0.12–28.2)     | <0.0001c|
| Death rate, cases per 100 patient-years       | 0.48                  | 0.42                 |         |
| Death rate, cases per 100 RRT-years           | 7.97                  | 4.63                 |         |
| **Clinical data**                             |                       |                      |         |
| Coronary artery disease, n (% of all)         | 174 (52.4)            | 168 (31.9)           | <0.0001c|
| Myocardial infarction, n (% of all)           | 98 (29.5)             | 101 (19.2)           | 0.009b  |
| Parathyroidectomy, n (% of all)               | 2 (0.60)              | 21 (3.98)            | 0.0009b |
| Treatment with cinacalcet hydrochloride       | 24 (7.2)              | 98 (18.6)            | <0.0001b|
| **Laboratory data**                           |                       |                      |         |
| Anti-HBc positive, n (% of all)               | 95 (26.0)             | 126 (23.9)           | 0.528b  |
| HBsAg positive, n (% of all anti-HBc positive)| 7 (7.4)               | 11 (8.7)             | 0.807b  |
| Anti-HCV positive, n (% of all)               | 26 (7.1)              | 57 (10.8)            | 0.062b  |
| HCV RNA positive, n (% of all anti-HCV positive)| 14 (53.8)         | 39 (68.4)            | 0.225b  |
| Responders to hepatitis B vaccine, n (% of all)| 202 (55.2)        | 315 (59.8)           | 0.191b  |
| 25(OH)D (ng/mL)a                             | 13.3 ± 3.9            | 14.5 ± 5.6           | 0.182a,d|
| Total calcium (mg/dL)                        | 8.83 ± 0.67           | 8.91 ± 0.82          | 0.264d  |
| Phosphates (mg/dL)                           | 5.03 ± 1.44           | 5.25 ± 1.49          | 0.054d  |
| PTH (pg/mL)                                  | 296 (12.9–3,757)      | 463 (12.7–3,741)     | <0.0001c|
| Total alkaline phosphatase (U/L)              | 98.2 (25.8–1,353)     | 97.1 (40.5–1,684)    | 0.528c  |

25(OH)D: 25-hydroxycholecalciferol, anti-HBc: antibodies to core antigen of hepatitis B virus, anti-HCV: antibodies to hepatitis C virus, HBsAg: surface antigen of hepatitis B virus, DM: diabetes mellitus, ESRD: end-stage renal disease, HCV RNA: ribonucleic acid of hepatitis C virus, PTH: parathyroid hormone, and RRT: renal replacement therapy.

A significant difference is indicated using bold font.

a n = 66 for type 2 DM nephropathy; n = 96 for other renal diseases.

b Fisher’s exact test.

c Mann-Whitney test.

d Unpaired t-test, Welch corrected.

this severe complication of type 2 DM. There is a link between vitamin D and T-cell functional balance: active form of vitamin D [1,25(OH)2D] has the inhibitory effect on the T helper (Th)17 and Th1 response [10]. Abnormalities in T-cell cytokine equilibrium [11–13] and plasma vitamin D concentrations [14–16] are related to cardiovascular events [13, 16] and immunononcompetence during infections [11, 14] and vaccinations [12, 15]. Serum PTH levels are dependent on serum vitamin D concentrations [17], and T cells are implicated in the mechanism of PTH action in bone [18].

Vitamin D activity may be adequately expressed if vitamin D pathway components (vitamin D binding protein, also referred to as group-specific component (GC), vitamin D receptor (VDR), and retinoid X receptors (RXRs)) are properly structured and regulated. The recent study by Zhang et al. [19] has shown that VDR BsmI polymorphism correlates with type 2 DM nephropathy and may be susceptible for early onset of this nephropathy. Among T-cell-related cytokine gene polymorphisms, promoter polymorphic variants of IL10 [20, 21] and IL6 [22] were already associated with the risk of type 2 DM nephropathy. Monocyte chemoattractant protein 1 (MCP-1) has been reported to participate in the pathogenesis of early type 2 DM nephropathy [23], but MCPI polymorphism in the promoter region was not differentially distributed between ESRD patients with type 2 DM nephropathy and healthy controls [24, 25].

To our knowledge, there are scarce data, if any, on ESRD due to type 2 DM nephropathy showing a frequency distribution of single nucleotide polymorphisms (SNPs) of T-cell-related IL genes: IL18 rs360719, IL12A rs568408, IL12B rs3212227, IL4R rs1805015, IL13 rs20541, IL28B rs8099917, and IL28B rs12979860 as well as vitamin D pathway genes: GC genes (GC rs2298849, rs7041, and rs1155563), VDR genes (VDR rs2228570, rs1544410), and RXRα genes (RXRA rs10776909, rs10881578, and rs749759). The aim of our study was to determine the potential association between aforementioned polymorphisms of T-cell-related cytokine genes and vitamin D pathway genes and ESRD due to type 2 DM nephropathy. For comparisons, aforementioned genotype frequencies of healthy controls as well as ESRD patients with other main causes of ESRD were used. Polymorphism related associations, if exist, could contribute to explanation of susceptibility to ESRD due to type 2 DM nephropathy and phenotype differences between ESRD patients with type 2 DM nephropathy and other causes of ESRD.
Table 3: Characteristics of hemodialysis patients grouped by a cause of ESRD.

| Parameter                          | Type 2 DM nephropathy (1) | Chronic glomerulonephritis (2) | Chronic tubulointerstitial nephritis (3) | Hypertensive nephropathy (4) | P value                        |
|------------------------------------|----------------------------|--------------------------------|-------------------------------------------|-------------------------------|--------------------------------|
| Demographic data                   |                            |                                |                                           |                               |                                |
| Male sex, n (% of all)             | 201 (54.9)                 | 110 (61.8)                     | 63 (53.4)                                 | 134 (58.0)                    | 0.386<sup>b</sup>              |
|                                    |                            |                                |                                           |                               |                                |
| Age at RRT beginning, years        | 62.9 ± 14.1                | 47.4 ± 17.6                    | 59.9 ± 16.6                               | 63.3 ± 13.6                   |                                |
|                                    |                            |                                |                                           |                               |                                |
| RRT duration, years                | 3.29 (0.06–28.0)           | 5.73 (0.16–28.2)               | 4.82 (0.33–26.5)                          | 3.82 (0.12–20.4)              |                                |
|                                    |                            |                                |                                           |                               |                                |
| Death rate, cases per 100 patient-years | 0.48                      | 0.41                           | 0.44                                      | 0.42                          |                                |
| Death rate, cases per 100 dialysis-years | 7.97                     | 2.87                           | 5.28                                      | 6.70                          |                                |
|                                      | n = 366                    | n = 178                        | n = 118                                   | n = 231                       |                                |
| Clinical data                      |                            |                                |                                           |                               |                                |
| Coronary artery disease, n (% of all) | 174 (52.4)               | 43 (24.2)                      | 29 (24.6)                                 | 96 (41.5)                     | <0.0001<sup>b</sup>           |
|                                    |                            |                                |                                           |                               |                                |
| Myocardial infarction, n (% of all) | 98 (29.5)                 | 25 (14.0)                      | 17 (14.4)                                 | 59 (25.5)                     | <0.0001<sup>b</sup>           |
|                                    |                            |                                |                                           |                               |                                |
| PTX, n (% of all)                  | 2 (0.60)                   | 14 (7.9)                       | 5 (4.2)                                   | 2 (0.87)                      | <0.0001<sup>b</sup>           |
|                                    |                            |                                |                                           |                               |                                |
| Treatment with cinacalcet hydrochloride | 24 (7.2)                | 48 (27.0)                      | 21 (17.8)                                 | 29 (12.6)                     | <0.0001<sup>b</sup>           |
|                                      | n = 366                    | n = 178                        | n = 118                                   | n = 231                       |                                |
| Laboratory data                    |                            |                                |                                           |                               |                                |
| Anti-HBc positive, n (% of all)     | 95 (26.0)                  | 53 (29.8)                      | 25 (21.2)                                 | 48 (20.8)                     | 0.233<sup>b</sup>             |
|                                    |                            |                                |                                           |                               |                                |
| HBsAg positive, n (% of all anti-HBc positive) | 7 (7.4)                 | 10 (18.9)                      | 0 (0.0)                                   | 1 (2.08)                      | 0.0006<sup>b</sup>            |
|                                    |                            |                                |                                           |                               |                                |
| Anti-HCV positive, n (% of all)     | 26 (7.1)                   | 33 (18.5)                      | 11 (9.3)                                  | 13 (5.6)                      | <0.0001<sup>b</sup>           |
|                                    |                            |                                |                                           |                               |                                |
| HCV RNA positive, n (% of all anti-HCV positive) | 14 (53.8)                | 27 (81.8)                      | 4 (36.4)                                  | 8 (61.5)                      | <0.0001<sup>b</sup>           |
Table 3: Continued.

| Parameter                        | Type 2 DM nephropathy (1) | Chronic glomerulonephritis (2) | Chronic tubulointerstitial nephritis (3) | Hypertensive nephropathy (4) | P value |
|----------------------------------|---------------------------|--------------------------------|----------------------------------------|-----------------------------|---------|
| Responders to hepatitis B vaccine, n (% of all) | 202 (55.2)                | 107 (60.1)                      | 70 (59.3)                              | 138 (59.7)                  | 0.598   |
| 25(OH)D (ng/mL)                 | 13.3 ± 3.9                | 14.2 ± 7.3                      | 15.7 ± 4.3                             | 14.1 ± 3.9                  | 0.453   |
| Total calcium (mg/dL)            | 8.83 ± 0.67               | 8.85 ± 0.85                     | 9.04 ± 0.61                            | 8.88 ± 0.87                 | 0.239   |
| Phosphates (mg/dL)              | 5.03 ± 1.44               | 5.63 ± 1.59                     | 4.92 ± 1.29                            | 5.15 ± 1.47                 |         |
| PTH (pg/mL)                     | 296 (12.9–3,757)          | 632 (12.7–3,118)                | 426 (45.8–3,741)                       | 364 (19.5–2,351)            |         |
| Total ALP (U/L)                 | 98.2 (25.8–1,353)         | 113 (44.5–860)                  | 89.0 (40.5–1,684)                      | 90.9 (41.0–1,110)           |         |

25(OH)D: 25-hydroxycholecalciferol, anti-HBC: antibodies to core antigen of hepatitis B virus, anti-HCV: antibodies to hepatitis C virus, HBsAg: surface antigen of hepatitis B virus, DM: diabetes mellitus, ESRD: end-stage renal disease, HCV RNA: ribonucleic acid of hepatitis C virus, PTH: parathyroid hormone, and RRT: renal replacement therapy.

a n = 66 for type 2 DM nephropathy, n = 40 for chronic glomerulonephritis, n = 13 for chronic interstitial nephritis, and n = 43 for hypertensive nephropathy.
b Chi squared test.
c Kruskal-Wallis test.
d ANOVA test.
e Fisher’s exact test.

2. Material and Methods

2.1. Patients and Controls. Blood samples for genotype analyses are collected since 2009 from ESRD patients (estimated glomerular filtration rate (eGFR) category G5 in accordance with KDIGO recommendations [26]). All subjects were treated with HD on enrolment. Controls were recruited from blood donors and healthy volunteers unrelated to patients. All enrolled individuals live/lived in the Greater Poland region of Poland.

Genotyping of IL18 rs360719, IL12A rs568408, IL12B rs3212227, IL4R rs1805015, and IL13 rs20541 polymorphisms was performed in 2009–2012 using currently available material. Results had been analyzed in our previous studies in the context of responsiveness to the surface antigen of hepatitis B virus (HBsAg) using data of all (not segregated) patients [27–30]. For this study, we used results of controls and patients with type 2 DM nephropathy, chronic glomerulonephritis, chronic infective tubulointerstitial nephritis, and hypertensive nephropathy.

IL28B rs8099917, IL28B rs12979860, GC rs2298849, GC rs7041, GC rs1155563, VDR rs2228570, VDR rs1544410, RXRA rs10776909, RXRA rs10881578, and RXRA rs749759 polymorphisms were analyzed in winter 2013/2014 among HD patients with ESRD (n = 893) due to type 2 DM nephropathy (n = 366), chronic glomerulonephritis (n = 178), chronic infective tubulointerstitial nephritis (n = 118), and hypertensive nephropathy (n = 231) as well as healthy controls (n = 378). DM was not diagnosed in patients having renal diseases other than type 2 DM nephropathy.

Healthy individuals and HD patients with other renal diseases as cause of ESRD served as reference groups for a frequency distribution of tested polymorphic variants. All examined subjects were of Caucasian race.

Basic clinical and laboratory data were collected on enrolment and they are updated every year.

2.2. Genotyping. Genomic DNA for genotype analysis was isolated from peripheral blood lymphocytes by salt-out extraction procedure.

Genotyping of IL18 rs360719, IL12A rs568408, IL12B rs3212227, IL4R rs1805015, and IL13 rs20541 polymorphisms was performed as previously described [27–30].

IL28B rs8099917 and IL28B rs12979860 polymorphisms were genotyped using high-resolution melting curve analysis (HRM) on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) with the use of 5x HOT FIREPol EvaGreen HRM Mix (Solis BioDyne, Tartu, Estonia). The PCR program consisted of an initial step at 95°C for 15 min to activate HOT FIREPol DNA polymerase, followed by 50 amplification cycles of denaturation at 95°C for 10 s, annealing at 61°C for 10 s, and elongation at 72°C for 15 s. Amplified DNA fragments were then subjected to HRM with 0.1°C increments in temperatures ranging from 76 to 96°C. Primers used for PCR with subsequent HRM analysis were as follows: rs8099917F 5’TTTGTCACI GTTCCCTCCTTGTG3’, rs8099917R 5’AAAGACATAA AAGCCAGCTACCA3’.
Table 4: Comparison of the distribution of polymorphic variants of tested genes between ESRD patients treated with hemodialysis due to type 2 DM nephropathy and healthy subjects.

| Parameter   | Type 2 DM nephropathy (frequency) | Healthy subjects (frequency) | Odds ratio (95% CI) | Two-tailed P | P_trend |
|-------------|-----------------------------------|-----------------------------|---------------------|--------------|---------|
| **IL18 rs360719** |                                   |                             |                     |              |         |
| TT          | 133 (0.54)                        | 121 (0.50)                  | Referent            | 0.233        |         |
| CT          | 102 (0.41)                        | 98 (0.41)                   | 0.947 (0.654–1.372) | 0.777        |         |
| CC          | 13 (0.05)                         | 21 (0.09)                   | 0.563 (0.270–1.174) | 0.145        |         |
| CT + CC     | 115 (0.46)                        | 119 (0.50)                  | 0.879 (0.616–1.254) | 0.526        |         |
| MAF         | 128 (0.26)                        | 140 (0.29)                  | 0.845 (0.638–1.119) | 0.268        |         |
| **IL12A rs568408** |                                   |                             |                     |              |         |
| G          | 173 (0.74)                        | 171 (0.71)                  | Referent            | 0.782        |         |
| A          | 52 (0.22)                         | 63 (0.26)                   | 0.816 (0.534–1.246) | 0.389        |         |
| A + G       | 61 (0.26)                         | 69 (0.29)                   | 0.874 (0.583–1.309) | 0.338        |         |
| MAF         | 70 (0.15)                         | 75 (0.16)                   | 0.976 (0.684–1.393) | 0.965        |         |
| **IL12B rs3212227** |                                   |                             |                     |              |         |
| A          | 156 (0.63)                        | 151 (0.63)                  | Referent            | 0.639        |         |
| C          | 84 (0.34)                         | 77 (0.32)                   | 1.056 (0.721–1.547) | 0.846        |         |
| C + A       | 91 (0.37)                         | 89 (0.37)                   | 0.990 (0.685–1.430) | 1.000        |         |
| MAF         | 98 (0.20)                         | 101 (0.21)                  | 0.927 (0.680–1.268) | 0.699        |         |
| **IL4 rs1805015** |                                   |                             |                     |              |         |
| T          | 205 (0.68)                        | 162 (0.72)                  | Referent            | 0.304        |         |
| C          | 82 (0.27)                         | 53 (0.24)                   | 1.223 (0.818–1.828) | 0.360        |         |
| C + T       | 16 (0.05)                         | 10 (0.04)                   | 1.264 (0.559–2.861) | 0.684        |         |
| MAF         | 114 (0.19)                        | 73 (0.16)                   | 1.197 (0.866–1.653) | 0.313        |         |
| **IL13 rs20541** |                                   |                             |                     |              |         |
| C          | 168 (0.55)                        | 124 (0.54)                  | Referent            | 0.457        |         |
| T          | 114 (0.38)                        | 84 (0.36)                   | 1.002 (0.695–1.443) | 1.000        |         |
| T + C       | 21 (0.07)                         | 22 (0.10)                   | 0.705 (0.371–1.338) | 0.324        |         |
| MAF         | 156 (0.26)                        | 128 (0.28)                  | 0.899 (0.684–1.182) | 0.489        |         |
| **IL28B rs8099917** |                                   |                             |                     |              |         |
| C          | 141 (0.42)                        | 164 (0.44)                  | Referent            | 0.504        |         |
| T          | 219 (0.65)                        | 245 (0.65)                  | Referent            | 0.504        |         |
| G          | 107 (0.31)                        | 123 (0.33)                  | 0.973 (0.709–1.336) | 0.872        |         |
| G + T       | 13 (0.04)                         | 7 (0.02)                    | 2.078 (0.814–5.302) | 0.169        |         |
| MAF         | 133 (0.20)                        | 137 (0.18)                  | 1.092 (0.837–1.423) | 0.560        |         |
| **IL28B rs12979860** |                                   |                             |                     |              |         |
| C          | 141 (0.42)                        | 164 (0.44)                  | Referent            | 0.669        |         |
| T          | 157 (0.47)                        | 166 (0.45)                  | 1.100 (0.804–1.505) | 0.576        |         |
| T + C       | 38 (0.11)                         | 42 (0.11)                   | 1.052 (0.643–1.723) | 0.900        |         |
| MAF         | 116 (0.29)                        | 250 (0.34)                  | 1.049 (0.842–1.307) | 0.713        |         |
| **GC rs2298849** |                                   |                             |                     |              |         |
| T          | 226 (0.62)                        | 237 (0.63)                  | Referent            | 0.250        |         |
| C          | 110 (0.30)                        | 124 (0.33)                  | 0.930 (0.679–1.274) | 0.688        |         |
| C + T       | 28 (0.08)                         | 14 (0.04)                   | 2.097 (1.077–4.086) | 0.035        |         |
| MAF         | 166 (0.23)                        | 152 (0.20)                  | 1.162 (0.907–1.490) | 0.262        |         |
Table 4: Continued.

| Parameter | Type 2 DM nephropathy (frequency) | Healthy subjects (frequency) | Odds ratio (95% CI) | Two-tailed P | P<sub>trend</sub> |
|-----------|----------------------------------|-----------------------------|---------------------|--------------|-----------------|
| GC rs7041 | n = 343                          | n = 361                     | Referent            |              |                 |
| GG        | 112 (0.33)                       | 116 (0.32)                  |                    |              | 0.572           |
| GT        | 163 (0.47)                       | 186 (0.52)                  | 0.908 (0.650–1.268) | 0.609        |
| TT        | 68 (0.20)                        | 59 (0.16)                   | 1.194 (0.773–1.844) | 0.440        |
| GT + TT   | 231 (0.67)                       | 245 (0.68)                  | 0.977 (0.712–1.339) | 0.936        |
| MAF       | 299 (0.44)                       | 304 (0.42)                  | 1.062 (0.860–1.312) | 0.612        |
| GC rs115563 | n = 362                        | n = 377                     | Referent            |              |                 |
| TT        | 180 (0.50)                       | 189 (0.50)                  |                    |              | 0.541           |
| CT        | 141 (0.39)                       | 155 (0.41)                  | 0.955 (0.703–1.297) | 0.815        |
| CC        | 41 (0.11)                        | 33 (0.09)                   | 1.305 (0.789–2.155) | 0.311        |
| CT + CC   | 182 (0.50)                       | 188 (0.50)                  | 1.017 (0.762–1.356) | 0.941        |
| MAF       | 223 (0.31)                       | 221 (0.29)                  | 1.074 (0.859–1.341) | 0.567        |
| VDR rs2228570 | n = 345                      | n = 371                     | Referent            |              |                 |
| CC        | 101 (0.29)                       | 103 (0.28)                  |                    |              | 0.401           |
| CT        | 175 (0.51)                       | 183 (0.49)                  | 0.975 (0.691–1.376) | 0.930        |
| TT        | 69 (0.20)                        | 85 (0.23)                   | 0.828 (0.544–1.260) | 0.394        |
| CT + TT   | 244 (0.71)                       | 268 (0.72)                  | 0.929 (0.671–1.285) | 0.679        |
| MAF       | 313 (0.45)                       | 353 (0.48)                  | 0.915 (0.743–1.126) | 0.432        |
| VDR rs1544410 | n = 359                  | n = 372                     | Referent            |              | 0.753           |
| GG        | 137 (0.38)                       | 148 (0.40)                  |                    |              |                 |
| AG        | 165 (0.46)                       | 165 (0.44)                  | 1.080 (0.787–1.483) | 0.686        |
| AA        | 57 (0.16)                        | 59 (0.16)                   | 1.044 (0.678–1.607) | 0.912        |
| AG + AA   | 222 (0.62)                       | 224 (0.60)                  | 1.071 (0.795–1.442) | 0.705        |
| MAF       | 279 (0.39)                       | 283 (0.38)                  | 1.035 (0.839–1.278) | 0.788        |
| RXRA rs10776909 | n = 364 | n = 378 | Referent | 0.426 |
| CC        | 233 (0.64)                       | 250 (0.66)                  |                    |              |                 |
| CT        | 111 (0.30)                       | 112 (0.30)                  | 1.063 (0.774–1.461) | 0.746        |
| TT        | 20 (0.05)                        | 16 (0.04)                   | 1.341 (0.679–2.651) | 0.490        |
| CT + TT   | 131 (0.36)                       | 128 (0.34)                  | 1.098 (0.812–1.485) | 0.590        |
| MAF       | 151 (0.21)                       | 144 (0.19)                  | 1.112 (0.862–1.435) | 0.452        |
| RXRA rs10881578 | n = 365 | n = 377 | Referent | 0.168 |
| AA        | 197 (0.54)                       | 183 (0.48)                  |                    |              |                 |
| AG        | 134 (0.37)                       | 154 (0.41)                  | 0.808 (0.775–1.046) | 0.185        |
| GG        | 34 (0.09)                        | 40 (0.11)                   | 0.790 (0.479–1.301) | 0.376        |
| AG + GG   | 168 (0.46)                       | 194 (0.51)                  | 0.804 (0.603–1.073) | 0.143        |
| MAF       | 202 (0.28)                       | 234 (0.31)                  | 0.850 (0.680–1.063) | 0.172        |
| RXRA rs749759 | n = 355                  | n = 370                     | Referent            |              | 0.850           |
| GG        | 207 (0.58)                       | 221 (0.60)                  |                    |              |                 |
| AG        | 125 (0.35)                       | 123 (0.33)                  | 1.085 (0.794–1.216) | 0.632        |
| AA        | 23 (0.06)                        | 26 (0.07)                   | 0.944 (0.522–1.708) | 0.881        |
| AG + AA   | 148 (0.42)                       | 149 (0.40)                  | 1.061 (0.789–1.426) | 0.706        |
| MAF       | 171 (0.24)                       | 175 (0.24)                  | 1.024 (0.804–1.304) | 0.894        |

ESRD: end-stage renal disease, DM: diabetes mellitus, and MAF: minor allele frequency.

*Not consistent with Hardy-Weinberg equilibrium.

rs12979860F 5’CGTGCCTGTCGTACTGAA3’, and rs12979860R 5’AGGCTCAGGTTCAATCAG3’.

Genotyping of the GC rs115563, GC rs2298849, RXRA rs10881578, and RXRA rs10776909 polymorphisms was carried out by HRM on the Bio-Rad CFX96 Real Time PCR system (Bio-Rad, Hercules, CA). DNA fragments amplified with the use of specific primers were subjected to HRM with 0.1°C increments in temperatures ranging from 71 to 92°C. Genotyping of the GC rs7041, RXRA rs749759, VDR rs1544410, and VDR rs2228570 was performed using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method according to the
Table 5: Comparison of the distribution of polymorphic variants of tested genes between ESRD patients treated with hemodialysis due to type 2 DM nephropathy and the most common causes of ESRD other than type 2 DM nephropathy (chronic glomerulonephritis, chronic tubulointerstitial nephritis, and hypertensive nephritis).

| Genotype     | Type 2 DM nephropathy (frequency) | Other causes of ESRD (frequency) | Odds ratio (95% CI) | Two-tailed P | P_trend |
|--------------|----------------------------------|---------------------------------|---------------------|--------------|---------|
| **IL18 rs360719** |                                  |                                 |                     |              |         |
| TT           | 133 (0.54)                       | 186 (0.53)                      | Referent            | —            | 0.362   |
| CT           | 102 (0.41)                       | 135 (0.38)                      | 1.057 (0.752–1.485) | 0.795        |         |
| CC           | 13 (0.05)                        | 32 (0.09)                       | 0.568 (0.287–1.124) | 0.107        |         |
| CT + CC      | 115 (0.46)                       | 167 (0.47)                      | 0.963 (0.696–1.334) | 0.868        |         |
| MAF          | 128 (0.26)                       | 199 (0.28)                      | 0.886 (0.684–1.149) | 0.370        |         |
| **IL12A rs568408** |                                  |                                 |                     |              |         |
| GG           | 173 (0.74)                       | 234 (0.69)                      | Referent            | —            | 0.303   |
| AG           | 52 (0.22)                        | 89 (0.26)                       | 0.790 (0.533–1.060) | 0.275        |         |
| AA           | 9 (0.04)                         | 14 (0.04)                       | 0.870 (0.368–2.055) | 0.831        |         |
| AG + AA      | 61 (0.26)                        | 103 (0.31)                      | 0.801 (0.552–1.163) | 0.260        |         |
| MAF          | 70 (0.15)                        | 117 (0.17)                      | 0.837 (0.606–1.157) | 0.319        |         |
| **IL12B rs3212227** |                                  |                                 |                     |              |         |
| AA           | 156 (0.63)                       | 205 (0.58)                      | Referent            | —            | 0.176   |
| AC           | 84 (0.34)                        | 132 (0.38)                      | 0.836 (0.593–1.068) | 0.337        |         |
| CC           | 7 (0.03)                         | 15 (0.04)                       | 0.613 (0.244–1.540) | 0.376        |         |
| AC + CC      | 91 (0.37)                        | 147 (0.42)                      | 0.814 (0.582–1.136) | 0.236        |         |
| MAF          | 98 (0.20)                        | 162 (0.23)                      | 0.828 (0.624–1.098) | 0.215        |         |
| **IL4R rs1805015** |                                  |                                 |                     |              |         |
| TT           | 205 (0.68)                       | 295 (0.68)                      | Referent            | —            | 0.871   |
| CT           | 82 (0.27)                        | 121 (0.28)                      | 0.975 (0.700–2.360) | 0.933        |         |
| CC           | 16 (0.05)                        | 20 (0.05)                       | 1.151 (0.583–2.275) | 0.728        |         |
| CT + CC      | 98 (0.32)                        | 141 (0.32)                      | 1.000 (0.731–1.368) | 1.000        |         |
| MAF          | 114 (0.19)                       | 161 (0.18)                      | 1.023 (0.784–1.335) | 0.919        |         |
| **IL13 rs20541** |                                  |                                 |                     |              |         |
| CC           | 168 (0.55)                       | 242 (0.56)                      | Referent            | —            | 0.902   |
| CT           | 114 (0.38)                       | 166 (0.38)                      | 0.989 (0.726–1.348) | 1.000        |         |
| TT           | 21 (0.07)                        | 28 (0.06)                       | 1.080 (0.594–1.967) | 0.878        |         |
| CT + TT      | 135 (0.45)                       | 194 (0.44)                      | 1.002 (0.746–1.346) | 1.000        |         |
| MAF          | 156 (0.26)                       | 222 (0.25)                      | 1.015 (0.800–1.287) | 0.950        |         |
| **IL28B rs8099917** |                                  |                                 |                     |              |         |
| TT           | 219 (0.65)                       | 317 (0.64)                      | Referent            | —            | 0.858   |
| GT           | 107 (0.31)                       | 162 (0.33)                      | 0.956 (0.709–1.289) | 0.820        |         |
| GG           | 13 (0.04)                        | 14 (0.03)                       | 1.344 (0.620–2.916) | 0.549        |         |
| GT + GG      | 120 (0.35)                       | 176 (0.36)                      | 0.987 (0.739–1.318) | 0.941        |         |
| MAF          | 133 (0.20)                       | 190 (0.19)                      | 1.022 (0.799–1.309) | 0.910        |         |
| **IL28B rs12979860** |                                 |                                 |                     |              |         |
| TT           | 226 (0.62)                       | 339 (0.65)                      | Referent            | —            | 0.952   |
| CT           | 110 (0.30)                       | 165 (0.31)                      | 1.000 (0.745–1.342) | 1.000        |         |
| CC           | 28 (0.08)                        | 20 (0.04)                       | 2.100 (1.155–3.819) | 0.014        |         |
| CT + CC      | 138 (0.38)                       | 185 (0.35)                      | 1.119 (0.848–1.477) | 0.436        |         |
| MAF          | 166 (0.23)                       | 205 (0.20)                      | 1.215 (0.964–1.530) | 0.111        |         |
| **IL28B rs2298849** |                                 |                                 |                     |              |         |
| TT           | 226 (0.62)                       | 339 (0.65)                      | Referent            | —            | 0.109   |
| CT           | 110 (0.30)                       | 165 (0.31)                      | 1.000 (0.745–1.342) | 1.000        |         |
| CC           | 28 (0.08)                        | 20 (0.04)                       | 2.100 (1.155–3.819) | 0.014        |         |
| CT + CC      | 138 (0.38)                       | 185 (0.35)                      | 1.119 (0.848–1.477) | 0.436        |         |
| MAF          | 166 (0.23)                       | 205 (0.20)                      | 1.215 (0.964–1.530) | 0.111        |         |
Table 5: Continued.

| Genotype | Type 2 DM nephropathy (frequency) | Other causes of ESRD (frequency) | Odds ratio (95% CI) | Two-tailed $P$ | $P_{\text{trend}}$ |
|----------|----------------------------------|---------------------------------|---------------------|---------------|------------------|
| GC rs7041 | $n = 343$                         | $n = 506$                       | Referent            |               | 0.247            |
| GG       | 112 (0.33)                        | 182 (0.36)                      | —                   |               |                  |
| GT       | 163 (0.47)                        | 236 (0.47)                      | 1.122 (0.824–1.528) | 0.480         |                  |
| TT       | 68 (0.20)                         | 88 (0.17)                       | 1.256 (0.846–1.863) | 0.267         |                  |
| GT + TT  | 231 (0.67)                        | 324 (0.64)                      | 1.159 (0.867–1.548) | 0.340         |                  |
| MAF      | 299 (0.44)                        | 412 (0.41)                      | 1.125 (0.923–1.369) | 0.259         |                  |
| GC rs155563 | $n = 362$                        | $n = 527$                       | Referent            |               | 0.614            |
| TT       | 180 (0.50)                        | 252 (0.48)                      | —                   |               |                  |
| CT       | 141 (0.39)                        | 213 (0.40)                      | 0.927 (0.696–1.234) | 0.610         |                  |
| CC       | 41 (0.11)                         | 62 (0.12)                       | 0.926 (0.597–1.435) | 0.740         |                  |
| CT + CC  | 182 (0.50)                        | 275 (0.52)                      | 0.927 (0.709–1.211) | 0.585         |                  |
| MAF      | 223 (0.31)                        | 337 (0.32)                      | 0.947 (0.772–1.161) | 0.638         |                  |
| VDR rs2228570 | $n = 345$                  | $n = 503$                       | Referent            |               | 0.541            |
| CC       | 101 (0.29)                        | 130 (0.26)                      | —                   |               |                  |
| CT       | 175 (0.51)                        | 275 (0.55)                      | 0.819 (0.594–1.130) | 0.249         |                  |
| TT       | 69 (0.20)                         | 98 (0.19)                       | 0.906 (0.606–1.356) | 0.682         |                  |
| CT + TT  | 244 (0.71)                        | 373 (0.74)                      | 0.842 (0.620–1.143) | 0.273         |                  |
| MAF      | 313 (0.45)                        | 471 (0.47)                      | 0.943 (0.776–1.145) | 0.588         |                  |
| VDR rs1544410 | $n = 359$                  | $n = 512$                       | Referent            |               | 0.598            |
| GG       | 137 (0.38)                        | 189 (0.37)                      | —                   |               |                  |
| AG       | 165 (0.46)                        | 235 (0.46)                      | 0.969 (0.720–1.303) | 0.880         |                  |
| AA       | 57 (0.16)                         | 88 (0.17)                       | 0.894 (0.599–1.332) | 0.613         |                  |
| AG + AA  | 222 (0.62)                        | 323 (0.63)                      | 0.948 (0.718–1.253) | 0.722         |                  |
| MAF      | 279 (0.39)                        | 411 (0.40)                      | 0.948 (0.778–1.152) | 0.626         |                  |
| RXRA rs10776909 | $n = 364$              | $n = 526$                       | Referent            |               | 0.298            |
| CC       | 233 (0.64)                        | 308 (0.59)                      | —                   |               |                  |
| CT       | 111 (0.30)                        | 196 (0.37)                      | 0.749 (0.561–0.999) | 0.050         |                  |
| TT       | 20 (0.05)                         | 22 (0.04)                       | 1.202 (0.641–2.254) | 0.629         |                  |
| CT + TT  | 131 (0.36)                        | 218 (0.41)                      | 0.794 (0.603–1.046) | 0.108         |                  |
| MAF      | 151 (0.21)                        | 240 (0.23)                      | 0.883 (0.702–1.112) | 0.317         |                  |
| RXRA rs10881578 | $n = 365$              | $n = 525$                       | Referent            |               | 0.134            |
| AA       | 197 (0.54)                        | 252 (0.48)                      | —                   |               |                  |
| AG       | 134 (0.37)                        | 220 (0.42)                      | 0.779 (0.586–1.035) | 0.096         |                  |
| GG       | 34 (0.09)                         | 53 (0.10)                       | 0.821 (0.513–1.312) | 0.478         |                  |
| AG + GG  | 168 (0.46)                        | 273 (0.52)                      | 0.787 (0.602–1.029) | 0.088         |                  |
| MAF      | 202 (0.28)                        | 326 (0.31)                      | 0.850 (0.690–1.046) | 0.139         |                  |
| RXRA rs749759 | $n = 355$              | $n = 514$                       | Referent            |               | 0.082            |
| GG       | 207 (0.58)                        | 265 (0.52)                      | —                   |               |                  |
| AG       | 125 (0.35)                        | 212 (0.41)                      | 0.755 (0.567–1.005) | 0.059         |                  |
| AA       | 23 (0.06)                         | 37 (0.07)                       | 0.796 (0.459–1.381) | 0.490         |                  |
| AG + AA  | 148 (0.42)                        | 249 (0.48)                      | 0.761 (0.579–1.000) | 0.053         |                  |
| MAF      | 171 (0.24)                        | 286 (0.28)                      | 0.823 (0.661–1.025) | 0.092         |                  |

ESRD: end-stage renal disease, DM: diabetes mellitus, and MAF: minor allele frequency.

*Not consistent with Hardy-Weinberg equilibrium.

manufacturer’s instructions (Fermentas, Vilnius, Lithuania). Primer sequences and conditions for HRM and PCR-RFLP analyses are presented in Table 1.

For quality control, the genotyping analysis was blinded to the subject’s case-control status. In addition, approximately 10% of the randomly chosen samples were regenotyped. Samples that failed the genotyping were excluded from further statistical analyses.

2.3. 25(OH)D Testing. Plasma 25(OH)D was determined in blindly selected 162 HD patients in the winter season of the year to avoid differences in sunlight exposure between patients who used to sunbathe and those who did not. Plasma 25(OH)D concentration was measured in HD patients who had not been treated with vitamin D or had stopped such a treatment for at least 3 weeks to obtain the so-called basic vitamin D concentrations. Under these conditions, there were
Table 6: Selected comparisons of the polymorphic variants distribution of tested genes between type 2 DM nephropathy patients, chronic infective tubulointerstitial nephritic patients, and healthy subjects.

| Genotype | Genotype frequencies | Odds ratio (95% CI) | Two-tailed $P$ | $P_{\text{trend}}$ |
|----------|----------------------|---------------------|---------------|-----------------|
| **Type 2 DM nephropathy versus chronic infective tubulointerstitial nephritis** | | | | |
| **IL18 rs360719** | | | | |
| $n=248$ | $n=77$ | Referent | | 0.033 |
| TT | 133 (0.54) | 54 (0.70) | 2.180 (1.217–3.905) | 0.009* |
| CT | 102 (0.41) | 19 (0.25) | 1.320 (0.412–4.228) | 0.783 |
| CC | 13 (0.05) | 4 (0.05) | 2.030 (1.173–3.512) | 0.012* |
| CT + CC | 115 (0.46) | 23 (0.30) | 1.636 (1.031–2.596) | 0.046 |
| **MAF** | 128 (0.26) | 27 (0.18) | 1.636 (1.031–2.596) | 0.046 |
| **Chronic infective tubulointerstitial nephritis versus healthy controls** | | | | |
| **IL18 rs360719** | | | | |
| $n=77$ | $n=240$ | Referent | | 0.005 |
| TT | 54 (0.70) | 121 (0.50) | 0.434 (0.242–0.781) | 0.006* |
| CT | 19 (0.25) | 98 (0.41) | 0.427 (0.140–1.303) | 0.160 |
| CC | 4 (0.05) | 21 (0.09) | 0.433 (0.250–0.750) | 0.004* |
| CT + CC | 23 (0.30) | 119 (0.50) | 0.516 (0.326–0.818) | 0.006 |

DM: diabetes mellitus; MAF: minor allele frequency.
Significant differences are indicated using bold font.
*Significant after the Bonferroni correction ($P < 0.017$).

no patients showing optimal plasma 25(OH)D levels (35–80 ng/mL for adults). To examine plasma 25(OH)D levels, a chemiluminescent microparticle immunoassay (CMIA) was used according to the manufacturer's instructions (Abbott Diagnostics ARCHITECT 25-OH VITAMIN D CMIA).

2.4. Statistical Methods. Results are presented as percentage for categorical variables, as mean with one standard deviation for normally distributed continuous variables or as median with range for not normally distributed continuous variables as tested by the Shapiro-Wilk test. Statistical tests used for comparison of data obtained in selected groups are indicated at $P$ values.

Hardy-Weinberg equilibrium (HWE) was tested to compare the observed genotype frequencies to the expected ones using Chi-square test. Distributions of tested polymorphisms were consistent with HWE with three exceptions which are indicated in tables showing analysis of genotype and allele distributions. The Fisher exact probability test or Chi-square test was used to evaluate deviations in genotype and allele prevalence between the examined groups. Homozygotes for the major allele were the reference group. The odds ratio (OR) with $P$ value and 95% confidence intervals (95% CI) value were calculated. All probabilities were two-tailed. Polymorphisms were tested for association using the Chi-square test for trend ($P_{\text{trend}}$). Power analysis was performed by Fisher's exact test.

Values of $P < 0.05$ were judged to be significant. However, associations were reported only if the following conditions were fulfilled.

1. A genotype distribution was consistent with HWE in a tested group and a referent group.
2. $P_{\text{trend}}$ was below 0.05.
3. Odds ratio remained significant after the Bonferroni correction applied for multiple testing, if appropriate.

Aforementioned statistical calculations were performed using GraphPad InStat 3.10, 32 bit for Windows, created on July 9, 2009 (GraphPad Software, Inc., La Jolla, USA), CytelStudio version 10.0, created on January 16, 2013 (CytelStudio Software Corporation, Cambridge, USA), and Statistica version 10, 2011 (StatSoft, Inc., Tulsa, USA).

3. Results

Characteristics of the examined HD patients are presented in Tables 2 and 3. ESRD patients due to type 2 DM nephropathy compared to non-DM ESRD patients showed older age at RRT onset, shorter treatment with RRT, higher death rate on RRT, higher prevalence of CAD and myocardial infarction, lower serum PTH level, and lower frequency of parathyroidectomy and treatment with cinacalcet.

In respect of the examined parameters, type 2 DM nephropathy patients differed the most significantly from chronic glomerulonephritic subjects, the least significantly from hypertensive nephropathy patients. There were no differences in frequency distributions of tested genotypes between type 2 DM nephropathy patients and healthy subjects (Table 4) as well as other ESRD patients analyzed together (Table 5) which could be judged as significant associations.

Comparisons of genotype and allele frequencies between type 2 DM nephropathy patients and other ESRD groups revealed associations only with chronic infective tubulointerstitial nephritic patients in respect of IL18 rs360719 (Table 6, no significant results are shown). Frequency of IL18 rs360719 allele C carriers was higher in type 2 DM nephropathy patients than in those with chronic infective tubulointerstitial...
Table 7: Comparison of the distribution of polymorphic variants of tested genes between ESRD patients treated with hemodialysis due to type 2 DM nephropathy grouped by diagnosis of CAD.

| Parameter | Type 2 DM nephropathy with CAD (frequency) | Type 2 DM nephropathy without CAD (frequency) | Odds ratio (95% CI) | Two-tailed P | P_trend |
|-----------|------------------------------------------|---------------------------------------------|-------------------|-------------|---------|
| **IL18 rs360719** | n = 124 | n = 109 | Referent | 0.269 |
| TT | 68 (0.55) | 53 (0.49) | Referent | 0.269 |
| CT | 51 (0.41) | 49 (0.45) | 1.128 (0.725–1.754) | 0.653 |
| CC | 5 (0.04) | 7 (0.06) | 0.628 (0.194–2.036) | 0.557 |
| CT + CC | 56 (0.45) | 56 (0.51) | 0.879 (0.560–1.380) | 0.645 |
| MAF | 61 (0.25) | 63 (0.29) | 0.803 (0.532–1.211) | 0.345 |
| **IL2A rs568408** | n = 117 | n = 102 | Referent | 0.361 |
| GG | 83 (0.71) | 77 (0.63) | Referent | 0.361 |
| AG | 28 (0.24) | 22 (0.22) | 1.181 (0.623–2.236) | 0.630 |
| AA | 6 (0.05) | 3 (0.03) | 1.855 (0.448–7.678) | 0.502 |
| AG + AA | 34 (0.29) | 25 (0.25) | 1.262 (0.691–2.304) | 0.542 |
| MAF | 40 (0.17) | 28 (0.14) | 1.311 (0.776–2.114) | 0.378 |
| **IL2B rs3122227** | n = 124 | n = 109 | Referent | 0.906 |
| AA | 78 (0.63) | 69 (0.63) | Referent | 0.906 |
| AC | 43 (0.35) | 36 (0.33) | 1.057 (0.611–1.829) | 0.889 |
| CC | 3 (0.02) | 4 (0.04) | 0.664 (0.143–3.069) | 0.708 |
| AC + CC | 46 (0.37) | 40 (0.37) | 1.017 (0.597–1.734) | 1.000 |
| MAF | 49 (0.20) | 44 (0.20) | 0.974 (0.618–1.535) | 0.909 |
| **IL13 rs20541** | n = 144 | n = 127 | Referent | 0.867 |
| CC | 80 (0.56) | 71 (0.56) | Referent | 0.867 |
| CT | 55 (0.38) | 46 (0.36) | 1.061 (0.640–1.759) | 0.898 |
| TT | 9 (0.06) | 10 (0.08) | 0.799 (0.307–2.077) | 0.808 |
| CT + TT | 64 (0.44) | 56 (0.44) | 1.014 (0.627–1.640) | 1.000 |
| MAF | 56 (0.19) | 50 (0.20) | 0.985 (0.644–1.504) | 0.944 |
| **IL28B rs8099917** | n = 163 | n = 145 | Referent | 0.752 |
| TT | 105 (0.66) | 97 (0.67) | Referent | 0.752 |
| GT | 52 (0.32) | 42 (0.29) | 1.144 (0.700–1.870) | 0.618 |
| GG | 6 (0.04) | 6 (0.04) | 0.924 (0.288–2.961) | 1.000 |
| GT + GG | 58 (0.36) | 48 (0.33) | 1.116 (0.697–1.819) | 0.719 |
| MAF | 64 (0.20) | 54 (0.20) | 0.967 (0.657–1.423) | 0.944 |
| **IL28B rs12979860** | n = 163 | n = 142 | Referent | 0.352 |
| CC | 69 (0.42) | 66 (0.46) | Referent | 0.352 |
| CT | 73 (0.45) | 62 (0.44) | 1.126 (0.698–1.816) | 0.715 |
| TT | 21 (0.13) | 14 (0.10) | 1.435 (0.674–3.055) | 0.448 |
| CT + TT | 94 (0.58) | 76 (0.54) | 1.183 (0.752–1.861) | 0.490 |
| MAF | 115 (0.35) | 90 (0.32) | 1.175 (0.838–1.647) | 0.396 |
| **GC rs2298849** | n = 172 | n = 158a | Referent | 0.173 |
| TT | 99 (0.58) | 106 (0.67) | Referent | 0.173 |
| CT | 60 (0.33) | 40 (0.25) | 1.606 (0.989–2.608) | 0.067 |
| CC | 13 (0.07) | 12 (0.08) | 1.160 (0.505–2.663) | 0.833 |
| CT + CC | 73 (0.42) | 52 (0.33) | 1.503 (0.959–2.355) | 0.088 |
| MAF | 166 (0.25) | 64 (0.20) | 1.313 (0.909–1.895) | 0.174 |
Table 7: Continued.

| Parameter | Type 2 DM nephropathy with CAD (frequency) | Type 2 DM nephropathy without CAD (frequency) | Odds ratio (95% CI) | Two-tailed $P$ | $P_{\text{trend}}$ |
|-----------|------------------------------------------|---------------------------------------------|-------------------|---------------|------------------|
| GC rs7041 | GG 57 (0.35) n = 161                      | GT 69 (0.43) n = 151                        | 1.327 (0.825–2.134) | 0.277         | 0.844            |
|           | TT 35 (0.22)                             | GT + TT 104 (0.65)                          | 1.061 (0.721–1.559) | 0.769         |                  |
|           | MAF 139 (0.43)                            |                                             | 1.025 (0.746–1.409) | 0.943         |                  |
| GC rs115563 | TT 82 (0.48) n = 172                    | GT 70 (0.41) n = 157                        | 1.106 (0.697–1.755) | 0.724         | 0.645            |
|           | CC 20 (0.12)                             | CT + CC 90 (0.52)                           | 1.112 (0.721–1.714) | 0.660         |                  |
|           | MAF 110 (0.32)                            |                                             | 1.084 (0.779–1.508) | 0.695         |                  |
| VDR rs2228570 | TT 43 (0.27) n = 162            | CT 93 (0.57) n = 152                        | 1.400 (0.829–2.363) | 0.230         | 0.316            |
|           | CC 26 (0.16)                             | CT + TT 119 (0.73)                          | 1.128 (0.688–1.849) | 0.705         |                  |
|           | MAF 145 (0.45)                            |                                             | 0.854 (0.624–1.169) | 0.365         |                  |
| VDR rs1544410 | GG 65 (0.38) n = 170          | AG 79 (0.46) n = 152                        | 1.030 (0.641–1.653) | 0.905         | 0.772            |
|           | AA 26 (0.15)                             | CT + TT 108 (0.71)                          | 1.109 (0.569–2.160) | 0.865         |                  |
|           | MAF 131 (0.39)                           |                                             | 1.048 (0.763–1.440) | 0.833         |                  |
| RXRA rs10776909 | CC 112 (0.65) n = 172       | CT 48 (0.28) n = 152                        | 0.948 (0.585–1.537) | 0.902         | 0.621            |
|           | TT 12 (0.07)                             | CT + TT 54 (0.34)                           | 1.032 (0.655–1.625) | 0.908         |                  |
|           | MAF 72 (0.21)                            |                                             | 1.107 (0.756–1.621) | 0.672         |                  |
| RXRA rs10881578 | AA 89 (0.51) n = 173       | AG 65 (0.38) n = 152                        | 1.268 (0.796–2.019) | 0.345         | 0.192            |
|           | GG 19 (0.11)                             | AG + GG 84 (0.49)                           | 1.316 (0.852–2.032) | 0.226         |                  |
|           | MAF 103 (0.30)                           |                                             | 1.272 (0.902–1.793) | 0.199         |                  |
| RXRA rs749759 | GG 100 (0.59) n = 169        | AG 59 (0.35) n = 152                        | 0.972 (0.610–1.551) | 1.000         | 0.812            |
|           | AA 10 (0.06)                             | AG + AA 69 (0.41)                           | 0.960 (0.615–1.496) | 0.910         |                  |
|           | MAF 79 (0.23)                            |                                             | 0.956 (0.665–1.375) | 0.882         |                  |

CAD: coronary artery disease, ESRD: end-stage renal disease, DM: diabetes mellitus, and MAF: minor allele frequency.

*Not consistent with Hardy-Weinberg equilibrium.

nephritis. The latter group showed lower frequency of $IL18$ rs360719 allele C carriers compared to healthy controls (Table 6).

Type 2 DM nephropathy patients with diagnosed CAD differed in tested genotype frequencies neither from type 2 DM nephropathy subjects without CAD (Table 7) nor from healthy controls (Table 8).

4. Discussion

Genetic studies involving DM nephropathy and related complications are not consistent in many aspects [31–34]. Some polymorphisms tested in this study were reported as being associated with type 1 DM ($IL12B$ rs3212227 [35], $IL4R$ [36, 37], $IL13$ [37], VDR rs1544410 [38, 39], and VDR
Table 8: Comparison of the distribution of polymorphic variants of tested genes between type 2 DM nephropathy patients with diagnosis of CAD and healthy controls.

| Parameter       | Type 2 DM nephropathy with CAD (frequency) | Healthy controls (frequency) | Odds ratio (95% CI) | Two-tailed P | P trend  |
|-----------------|-------------------------------------------|------------------------------|---------------------|--------------|----------|
| ***IL18*** rs360719 |                                           |                              |                     |              |          |
| TT              | 68 (0.55)                                 | 121 (0.50)                   | Referent            | 0.186        |          |
| CT              | 51 (0.41)                                 | 98 (0.41)                    | 0.926 (0.590–1.543) | 0.819        |          |
| CC              | 5 (0.04)                                  | 21 (0.09)                    | 0.424 (0.153–1.174) | 0.122        |          |
| CT + CC         | 56 (0.45)                                 | 119 (0.50)                   | 0.837 (0.542–1.294) | 0.440        |          |
| MAF             | 61 (0.25)                                 | 140 (0.29)                   | 0.792 (0.558–1.124) | 0.223        |          |
| ***IL12A*** rs568408 |                                         |                              |                     |              |          |
| GG              | 83 (0.71)                                 | 171 (0.71)                   | Referent            | 0.626        |          |
| AG              | 28 (0.24)                                 | 63 (0.26)                    | 0.916 (0.546–1.535) | 0.794        |          |
| AA              | 6 (0.05)                                  | 6 (0.03)                     | 2.060 (0.645–6.583) | 0.348        |          |
| AG + AA         | 34 (0.29)                                 | 69 (0.29)                    | 1.015 (0.624–1.653) | 1.000        |          |
| MAF             | 40 (0.17)                                 | 75 (0.16)                    | 1.113 (0.731–1.695) | 0.695        |          |
| ***IL12B*** rs3212227 |                                       |                              |                     |              |          |
| AA              | 78 (0.63)                                 | 151 (0.63)                   | Referent            | 0.475        |          |
| AC              | 43 (0.35)                                 | 77 (0.32)                    | 1.081 (0.681–1.717) | 0.813        |          |
| CC              | 3 (0.02)                                  | 12 (0.05)                    | 0.484 (0.133–1.766) | 0.397        |          |
| AC + CC         | 46 (0.37)                                 | 89 (0.37)                    | 1.001 (0.639–1.567) | 1.000        |          |
| MAF             | 49 (0.20)                                 | 101 (0.21)                   | 0.924 (0.631–1.354) | 0.757        |          |
| ***IL4R*** rs1805015 |                                      |                              |                     |              |          |
| TT              | 95 (0.66)                                 | 162 (0.72)                   | Referent            | 0.285        |          |
| CT              | 42 (0.29)                                 | 53 (0.24)                    | 1.351 (0.838–2.179) | 0.221        |          |
| CC              | 7 (0.05)                                  | 10 (0.04)                    | 1.194 (0.440–3.240) | 0.798        |          |
| CT + CC         | 49 (0.34)                                 | 63 (0.28)                    | 1.326 (0.845–2.083) | 0.246        |          |
| MAF             | 56 (0.19)                                 | 73 (0.16)                    | 1.247 (0.848–1.832) | 0.305        |          |
| ***IL13*** rs20541 |                                       |                              |                     |              |          |
| CC              | 80 (0.56)                                 | 124 (0.54)                   | Referent            | 0.469        |          |
| CT              | 55 (0.38)                                 | 84 (0.36)                    | 1.015 (0.653–1.578) | 1.000        |          |
| TT              | 9 (0.06)                                  | 22 (0.10)                    | 0.634 (0.278–1.447) | 0.324        |          |
| CT + TT         | 64 (0.44)                                 | 106 (0.46)                   | 0.936 (0.616–1.422) | 0.831        |          |
| MAF             | 73 (0.19)                                 | 73 (0.16)                    | 0.881 (0.630–1.231) | 0.510        |          |
| ***IL28B*** rs8099917 |                                    |                              |                     |              |          |
| TT              | 105 (0.64)                                | 245 (0.65)                   | Referent            | 0.584        |          |
| GT              | 52 (0.32)                                 | 123 (0.33)                   | 0.986 (0.663–1.467) | 1.000        |          |
| GG              | 6 (0.04)                                  | 7 (0.02)                     | 2.000 (0.656–6.094) | 0.229        |          |
| GT + GG         | 58 (0.36)                                 | 130 (0.35)                   | 1.041 (0.709–1.530) | 0.845        |          |
| MAF             | 64 (0.20)                                 | 137 (0.18)                   | 1.093 (0.786–1.521) | 0.658        |          |
| ***IL28B*** rs12979860 |                                  |                              |                     |              |          |
| CC              | 69 (0.42)                                 | 164 (0.44)                   | Referent            | 0.281        |          |
| CT              | 73 (0.45)                                 | 166 (0.45)                   | 1.045 (0.705–1.549) | 0.841        |          |
| TT              | 21 (0.13)                                 | 42 (0.11)                    | 1.188 (0.656–2.154) | 0.644        |          |
| CT + TT         | 94 (0.58)                                 | 208 (0.56)                   | 1.074 (0.740–1.558) | 0.776        |          |
| MAF             | 115 (0.35)                                | 250 (0.34)                   | 1.077 (0.819–1.416) | 0.644        |          |
| ***GC*** rs2298849 |                                    |                              |                     |              |          |
| TT              | 99 (0.58)                                 | 237 (0.63)                   | Referent            | 0.080        |          |
| CT              | 60 (0.35)                                 | 124 (0.33)                   | 1.158 (0.786–1.706) | 0.486        |          |
| CC              | 13 (0.07)                                 | 14 (0.04)                    | 2.223 (1.008–4.901) | 0.052        |          |
| CT + CC         | 73 (0.42)                                 | 138 (0.37)                   | 1.266 (0.876–1.830) | 0.220        |          |
| MAF             | 166 (0.25)                                | 152 (0.20)                   | 1.311 (0.969–1.774) | 0.092        |          |
| Parameter          | Type 2 DM nephropathy with CAD (frequency) | Healthy controls (frequency) | Odds ratio (95% CI) | Two-tailed P | P_{trend} |
|-------------------|-------------------------------------------|------------------------------|---------------------|--------------|-----------|
| GC rs7041         |                                            |                              |                     |              |           |
| GG                | 57 (0.35) (n = 161)                       | 116 (0.32) (n = 361)         | Referent            |              | 0.748     |
| GT                | 69 (0.43)                                 | 186 (0.52)                   | 0.755 (0.496–1.150) | 0.196        |           |
| TT                | 35 (0.22)                                 | 59 (0.16)                    | 1.207 (0.714–2.040) | 0.502        |           |
| GT + TT           | 104 (0.65)                                | 245 (0.68)                   | 0.864 (0.584–1.278) | 0.482        |           |
| MAF               | 139 (0.43)                                | 304 (0.42)                   | 1.044 (0.801–1.362) | 0.800        |           |
| GT + TT           | 104 (0.65)                                | 245 (0.68)                   | 0.864 (0.584–1.278) | 0.482        |           |
| GC rs115563       |                                            |                              |                     |              | 0.378     |
| TT                | 82 (0.48)                                 | 189 (0.50)                   | Referent            |              |           |
| CT                | 70 (0.41)                                 | 155 (0.41)                   | 1.041 (0.710–1.527) | 0.845        |           |
| CC                | 20 (0.12)                                 | 33 (0.09)                    | 1.397 (0.757–2.578) | 0.332        |           |
| CT + CC           | 90 (0.52)                                 | 188 (0.50)                   | 1.103 (0.769–1.583) | 0.646        |           |
| MAF               | 110 (0.32)                                | 221 (0.29)                   | 1.134 (0.861–1.494) | 0.411        |           |
| VDR rs2228570     |                                            |                              |                     |              |           |
| CC                | 43 (0.27)                                 | 103 (0.28)                   | Referent            |              | 0.386     |
| CT                | 93 (0.57)                                 | 183 (0.49)                   | 1.217 (0.788–1.880) | 0.384        |           |
| TT                | 26 (0.16)                                 | 85 (0.23)                    | 0.733 (0.416–1.290) | 0.321        |           |
| CT + TT           | 119 (0.73)                                | 268 (0.72)                   | 1.064 (0.702–1.613) | 0.833        |           |
| MAF               | 145 (0.45)                                | 353 (0.48)                   | 0.893 (0.687–1.160) | 0.434        |           |
| VDR rs1544410     |                                            |                              |                     |              |           |
| GG                | 65 (0.38)                                 | 148 (0.40)                   | Referent            |              | 0.880     |
| AG                | 79 (0.46)                                 | 165 (0.44)                   | 1.090 (0.734–1.620) | 0.687        |           |
| AA                | 26 (0.15)                                 | 59 (0.16)                    | 1.003 (0.581–1.732) | 1.000        |           |
| AG + AA           | 105 (0.62)                                | 224 (0.60)                   | 1.067 (0.735–1.549) | 0.776        |           |
| MAF               | 131 (0.39)                                | 283 (0.38)                   | 1.021 (0.784–1.329) | 0.931        |           |
| RXRA rs10776909   |                                            |                              |                     |              | 0.483     |
| CC                | 112 (0.65)                                | 250 (0.66)                   | Referent            |              |           |
| CT                | 48 (0.28)                                 | 112 (0.30)                   | 0.957 (0.638–1.434) | 0.838        |           |
| TT                | 12 (0.07)                                 | 16 (0.04)                    | 1.674 (0.767–3.656) | 0.209        |           |
| CT + TT           | 60 (0.35)                                 | 128 (0.34)                   | 1.046 (0.716–1.529) | 0.846        |           |
| MAF               | 72 (0.21)                                 | 144 (0.19)                   | 1.125 (0.819–1.545) | 0.518        |           |
| RXRA rs10881578   |                                            |                              |                     |              |           |
| AA                | 89 (0.51)                                 | 183 (0.48)                   | Referent            |              | 0.682     |
| AG                | 65 (0.38)                                 | 154 (0.41)                   | 0.868 (0.591–1.275) | 0.494        |           |
| GG                | 19 (0.11)                                 | 40 (0.11)                    | 0.977 (0.535–1.783) | 1.000        |           |
| AG + GG           | 84 (0.49)                                 | 194 (0.51)                   | 0.890 (0.621–1.276) | 0.582        |           |
| MAF               | 103 (0.30)                                | 234 (0.31)                   | 0.942 (0.714–1.243) | 0.725        |           |
| RXRA rs749759     |                                            |                              |                     |              |           |
| GG                | 100 (0.59)                                | 221 (0.60)                   | Referent            |              | 0.924     |
| AG                | 59 (0.35)                                 | 123 (0.33)                   | 1.060 (0.718–1.566) | 0.842        |           |
| AA                | 10 (0.06)                                 | 26 (0.07)                    | 0.850 (0.395–1.830) | 0.710        |           |
| AG + AA           | 69 (0.41)                                 | 149 (0.40)                   | 1.023 (0.707–1.482) | 0.925        |           |
| MAF               | 79 (0.23)                                 | 175 (0.24)                   | 0.985 (0.727–1.334) | 0.983        |           |

CAD: coronary artery disease, DM: diabetes mellitus, and MAF: minor allele frequency.
rs2228570 [38]), type 2 DM susceptibility (VDR rs2228570
[40], VDR rs1544410 [41]), and phenotype of type 2 DM (VDR
rs2228570 [42], VDR rs1544410 [41, 43]). VDR rs2228570
and IL4 polymorphisms were also related to the risk of
chronic kidney disease [44, 45]. On the other hand, there are
also data indicating no major effect of IL12B on type 1 DM
susceptibility in the entire study group [46], no association of
IL4R with type 1 DM [47], no evident causal relationship
between vitamin D pathway genes and type 2 DM, myocardial
infarction or mortality [48], similar distribution of genotypes,
allele and haplotypes of VDR rs2228570 and VDR rs731236
between type 2 DM patients and controls [49], no contribu-
tion of VDR rs1544410 to type 1 DM susceptibility [50], and
no association of VDR rs1544410 with chronic kidney disease
susceptibility [51].

In this study we were not able to show significant
differences in the frequency distribution of tested polymor-
phic variants of T-cell-related cytokine genes or vitamin D
pathway genes between HD patients with ESRD due to type
2 DM nephropathy and controls as well as HD patients
with other causes of ESRD analyzed together. This lack of
association was present although the examined type 2 DM
nephropathy patients showed clinical complications more
frequently than HD patients with other renal diseases: higher
dialysis related mortality rate [3], higher prevalence of CAD
including myocardial infarction [4], lower serum PTH, and
lower frequency of parathyroidectomy and treatment with
cinacalcet, all of them predictive for higher tendency to
adynamic bone disease [7]. Type 2 DM nephropathy patients
with or without diagnosis of CAD also did not differ in tested
genotype distributions.

Development of ESRD substantially ameliorates interpa-

tient clinical variability related to underlying renal impair-
ment and exposes uremia-related signs and symptoms. Com-
parisons of type 2 DM nephropathy patients in respect of
tested genotype frequencies with subjects showing other
common causes of ESRD revealed that the former group has
a higher IL18 rs360719 minor allele frequency than chronic
infective tubulointerstitial nephritic group. In this case, lower
IL18 rs360719 minor allele frequency in tubulointerstitial
nephritic patients was observed also when their results were
compared to those of healthy subjects. Sánchez et al. [52] have
found a significant increase in the relative expression of IL-
18 mRNA in individuals carrying the rs360719 minor allele.
IL-18 is IFN-γ inducing factor. Infective tubulointerstitial
nephritic patients are known to have diminished ability of
blood leukocytes to produce IFN-γ [53]. Our study indicates
that this may be related to lower frequency of IL18 rs360719
minor allele in this group compared to controls and type 2
DM nephropathy patients. In type 2 DM patients with overt
nephropathy, positive correlations between plasma IFN-γ,
proteinuria, and eGFR were found [54].

Due to limited financial support, we did not perform any
functional studies regarding T-cell-related interleukin and
vitamin D pathway genes, especially that multiple influences
independent or dependent on genetic profile need to be taken
into account in such studies conducted in the uremic milieu.
Although the examined patients showing ESRD due to type
2 DM nephropathy were well-defined group, they obviously
were not consistent in HLA DRB1 alleles. The latter could be
important in modulating susceptibility to advanced type 2
DM nephropathy and related complications, like it was shown
for type 1 DM [55] or type 2 DM [41], regardless of their
complications.

5. Summary

Distributions of tested T-cell cytokine gene polymorphisms
or vitamin D pathway gene polymorphisms are not signifi-
cantly different among patients with ESRD due to type 2 DM
nephropathy and healthy individuals. Subjects with ESRD
due to type 2 DM nephropathy differ in clinical manifesta-
tion from patients with other nephropathies leading to dialysis
dependency, but differences in tested genotype distributions
were found only in IL18 rs360719 compared with chronic
tubulointerstitial nephritic patients. This difference probably
arose from the fact that pathology of chronic infective
tubulointerstitial nephritis might have been associated with
this specific polymorphism.

6. Conclusions

In Polish HD patients, T-cell cytokine gene polymorphisms
and vitamin D pathway gene polymorphisms are not associ-
ated with ESRD due to type 2 DM nephropathy. IL18
polymorphism is worthy to be further investigated in chronic
infective tubulointerstitial nephritic patients as being possibly
associated with this disease.

Conflict of Interests

The authors declare that there is no conflict of interests
regarding the publication of this paper.

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