Association of Retinoid X Receptor Alpha Gene Polymorphism with Clinical Course of Chronic Glomerulonephritis

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Background:
Vitamin D (VD), VD binding protein, VD receptor (VDR), and retinoids are involved in pathogenesis of chronic glomerulonephritis (ChGN). We aimed to compare distribution of VD pathway gene polymorphisms in ChGN patients showing glomerular filtration rate (GFR) category 1-3, GFR category 5D, and healthy controls in order to elucidate the role of VD-related polymorphisms in the course of ChGN.

Material/Methods:
GFR category 1–3 ChGN patients (n=195), GFR category 5D ChGN patients (n=178), and controls (n=751) underwent testing for polymorphisms of genes encoding VD binding protein (GC, rs2298849, rs7041, rs1155563), VDR (VDR, rs2228570, rs1544410), and retinoid X receptor alpha (RXRA, rs10776909, rs10881578, rs749759).

Results:
Among GFR 1–3 subjects possessing TT genotype of RXRA rs10776909, 75% of patients had nephrotic syndrome, and 37.5% had glomerular hyperfiltration defined as GFR >140 ml/min/1.73 m², and, consequently, serum creatinine was lower in these patients compared to the remaining subjects (0.67±0.26 vs. 0.94±0.34, P=0.014). In GFR category 5D ChGN patients, frequencies of RXRA rs10776909 allele T (25% vs. 19%) and CT+TT (46% vs. 34%) were higher compared to frequencies of respective variants in controls (P=0.004, P=0.008).

Conclusions:
RXRA rs10776909 allele T is specifically involved in the pathogenesis of ChGN. This risk allele may be also associated with worse clinical course of ChGN.

MeSH Keywords: Glomerulonephritis • Kidney Failure, Chronic • Retinoid X Receptor alpha • Vitamin D

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895249
Background

Experimental and clinical studies provide evidence that vitamin D (VD), VD binding protein (also referred to as a group-specific component [GC]), VD receptor (VDR), and retinoids may be involved in the pathogenesis of chronic glomerulonephritis (ChGN) [1–7]. 1,25(OH)2D3 administered to subtotally nephrectomized rats caused less podocyte injury, decreased podocyte loss, and abrogation of podocyte hypertrophy compared to rats receiving solvent (ethanol) [1]. This active VD reduced glomerular hypercellularity and inflammatory infiltration in anti-Thy-1.1 nephritic rats [2]. 1,25(OH)2D3 was also capable of protecting human cultured podocytes from injury [3]. Increased urinary excretion of VD binding protein was shown in patients with more severe IgA nephropathy [4]. In uremic rats treated with VDR activator (paricalcitol), proteinuria decreased by 32%, glomerulosclerosis and interstitial infiltration were less intense, and renal oxidative stress was reduced compared to uremic rats receiving vehicle (propylene glycol) [5]. Gene expression of retinoid X receptor (RXR) alpha was markedly higher in glomeruli of chronic glomerulonephritic than non-nephritic rats [6]. Retinoids regulated the repair process of the podocytes in puromycin aminonucleoside-induced nephritis in rats [7].

We have attempted to compare distribution of VD pathway gene polymorphisms in ChGN patients showing glomerular filtration rate (GFR) category 1–3, ChGN patients treated with maintenance hemodialysis (HD), and healthy controls to elucidate the role of VD-related polymorphisms in the course of ChGN.

Material and Methods

Patients and controls

The study was conducted in the Department of Nephrology, Transplantology and Internal Diseases, Poznan University of Medical Sciences (PUMS), Poznan, Poland. ChGN patients with GFR category 1–3 (n=195) and ChGN patients showing GFR category 1–3 and 40 subjects currently treated with HD. In all patients, renal biopsy had been performed when patients showed GFR category 1-3a. In ChGN patients showing GFR category 1–3, the main histological finding was mesangial proliferative glomerulonephritis (MesPGN, n=125, 65.4% of 191 diagnostic results). Out of 195 ChGN patients with GFR category 1–3, 99 (50.8%) had nephrotic syndrome in the course of ChGN. In dialyzed ChGN patients, 36 out of 40 biopsy results were diagnostic and revealed mainly MesPGN (n=17, 47.2% of 36 diagnostic results).

Healthy volunteers from the Wielkopolska region of Poland (mainly blood donors) served as controls. Their characteristics are presented in Table 1.

All enrolled subjects underwent testing for polymorphisms of genes encoding VD binding protein (GC, rs2298849, rs7041, rs1155563), VDR (VDR, rs2228570, rs1544410), and RXR alpha (RXRA, rs10776909, rs10881578, rs749759).

Genotyping

Genotyping was performed in the Department of Biochemistry and Molecular Biology, PUMS, Poznan, Poland.

Genomic DNA for genotype analysis was isolated from peripheral blood lymphocytes by salt-out extraction procedure. Genotyping of the GC rs1155563, GC rs2298849, RXRA rs10881578, and RXRA rs10776909 polymorphisms was carried out by high-resolution melting curve analysis (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 manufacturer’s instructions (Fermentas, Vilnius, Lithuania). Primer sequences and conditions for HRM and PCR-RFLP analyses are presented in Supplementary material online, Table 1. For quality control, approximately 10% of the randomly chosen samples were re-genotyped. Samples with ambiguous results were excluded from further statistical analyses.

Statistical methods

The chi-square test was used to check Hardy-Weinberg equilibrium (HWE). Power analysis was performed by Fisher exact test.

For continuous variables, the Mann-Whitney test, t test, or Cochran-Cox test was used, as appropriate. Polymorphisms were tested for association with ChGN using the Cochran-Armitage trend test (P_trend). Genotype distributions were compared...
between cases and controls by standard χ² test \( (P_{\text{genotyping}}) \). Both \( P_{\text{trend}} \) and \( P_{\text{genotyping}} \) should be below 0.05 for significance.

Odds ratios (ORs) with 95% confidence intervals (95% CIs) were used to assess the strength of the association. Three inheritance models (dominant, recessive, and additive) were analyzed. All probabilities were 2-tailed. The \( P \) values with the Bonferroni correction for multiple testing were considered significant if a \( P \) value was lower than 0.017 (1 SNP, 3 models, 1 phenotype). Age and sex were covariates used for an adjustment.

Statistical analysis was performed using Graph-Pad InStat 3.10, 32 bit for Windows, created July 9, 2009 (GraphPad Software, Inc., San Diego, California, United States), CytelStudio version 10.0, created January 16, 2013 (CytelStudio Software Corporation, Cambridge, Massachusetts, United States), and Statistica version 10, 2011 (Stat Soft, Inc., Tulsa, Oklahoma, United States).

**Ethical approval**

The research design was approved by the Institutional Review Board of Poznan University of Medical Sciences, Poland. Written informed consent was obtained from all study participants.

**Results**

In all examined groups, all tested polymorphisms were in accordance with HWE. The only polymorphism associated with ChGN was that of RXRA rs10776909.

In GFR category 1–3 ChGN patients, the distribution of individuals showing nephrotic syndrome in the course of ChGN was not statistically different from RXRA rs10776909 polymorphic variant. GFR differences did not reach statistical significance. However, among GFR 1–3 subjects possessing TT genotype of RXRA rs10776909 (n=8), 75% of patients had nephrotic syndrome, and 37.5% of individuals revealed minimal change disease. Glomerular hyperfiltration defined as GFR over 140 ml/min/1.73 m² [8,9] was shown in 37.5% of TT subjects (all had nephrotic syndrome), and serum creatinine concentration was significantly lower in all these patients compared to the remaining subjects (Table 4).

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GFR category 1–3 ChGN patients, showing the most frequently occurring histological type of ChGN – MesGN – did not differ in distribution of VD pathway gene polymorphisms from controls (Table 2) or GFR category 5D ChGN subjects (Table 3). In this group, frequencies of CC, CT, CT+TT, and minor allele frequency (MAF) of RXRA rs10776909 were between those shown in controls and GFR category 5D ChGN individuals. A significant trend for decreasing frequency of CC genotype with concomitant increasing frequency of allele T was demonstrated in tested groups categorized with accordance to GFR (controls – normal GFR, GFR category 1–3 ChGN patients – moderately decreased GFR, HD patients – severe GFR deterioration) (Figure 1).

### Table 1. Characteristics of ChGN patients and healthy volunteers.

| Parameter               | ChGN patients |                  | Healthy volunteers |
|-------------------------|---------------|------------------|--------------------|
|                         | GFR category 1–3 | GFR category 5D | N=751              |
| Age, years              | 35, 18–76     | 56, 17–88       | 45, 18–70         |
| Age at RRT onset, years | –             | 48, 11–84       | –                  |
| Males, n,% of all       | 72, 37%       | 110, 62%        | 541, 72%          |
| RRT modality            | –             | IHD             | –                  |
| Serum creatinine, mg/dl | 0.82, 0.35–2.24 | 6.15, 3.1–9.2 | Na                |
| eGFR, ml/min/1.73 m²    | 91, 32–243    | –               | –                  |
| eKt/V                   | –             | 1.30, 0.54–1.91 | –                  |

Data are presented as median (minimum - maximum), or number (percentage). Conversion factor to SI units for creatinine is: 1 mg/dl=88.4 µmol/l. ChGN – chronic glomerulonephritis; eKt/V – equilibrated dialysis clearance by distribution volume; GFR – glomerular filtration rate; IHD – intermittent hemodialysis; Na – not available; RRT – renal replacement therapy.

### Table 2. Characteristics of ChGN patients and healthy volunteers.

| Parameter               | ChGN patients |                  | Healthy volunteers |
|-------------------------|---------------|------------------|--------------------|
|                         | GFR category 1–3 | GFR category 5D | N=751              |
| Age, years              | 35, 18–76     | 56, 17–88       | 45, 18–70         |
| Age at RRT onset, years | –             | 48, 11–84       | –                  |
| Males, n,% of all       | 72, 37%       | 110, 62%        | 541, 72%          |
| RRT modality            | –             | IHD             | –                  |
| Serum creatinine, mg/dl | 0.82, 0.35–2.24 | 6.15, 3.1–9.2 | Na                |
| eGFR, ml/min/1.73 m²    | 91, 32–243    | –               | –                  |
| eKt/V                   | –             | 1.30, 0.54–1.91 | –                  |
Table 2. Comparison of the distribution of VD pathway gene polymorphisms between ChGN patients showing GFR category 1–3 and healthy subjects.

| Genotype     | ChGN GFR category 1–3 (frequency) | Healthy subjects (frequency) | Odds ratio (95%CI) | Two-tailed P | $P_{trend}$ | $P_{genotyping}$ |
|--------------|-----------------------------------|------------------------------|--------------------|--------------|------------|-----------------|
| GC rs2298849 | n=193                             | n=748                        |                    |              |            |                 |
| TT           | 123 (0.64)                        | 461 (0.62)                   | Reference          | –            | 0.5        | 0.8             |
| CT           | 64 (0.33)                         | 257 (0.34)                   | 0.9333 (0.654–1.325) | 0.8          |            |                 |
| CC           | 6 (0.03)                          | 30 (0.04)                    | 0.750 (0.250–1.886) | 0.7          |            |                 |
| CT+CC        | 70 (0.36)                         | 287 (0.38)                   | 0.914 (0.648–1.284) | 0.7          |            |                 |
| MAF          | 0.20                              | 0.21                         | 0.912 (0.680–1.214) | 0.6          |            |                 |
| GC rs7041    | n=193                             | n=728                        |                    |              |            |                 |
| GG           | 63 (0.33)                         | 244 (0.34)                   | Reference          | –            | 0.4        | 0.4             |
| GT           | 90 (0.46)                         | 362 (0.50)                   | 0.963 (0.662–1.407) | 0.9          |            |                 |
| TT           | 40 (0.21)                         | 122 (0.17)                   | 1.270 (0.784–2.039) | 0.4          |            |                 |
| GT+TT        | 130 (0.67)                        | 484 (0.66)                   | 1.040 (0.734–1.485) | 0.9          |            |                 |
| MAF          | 0.44                              | 0.42                         | 1.104 (0.874–1.393) | 0.4          |            |                 |
| GC rs1155563 | n=193                             | n=748                        |                    |              |            |                 |
| TT           | 97 (0.50)                         | 355 (0.47)                   | Reference          | –            | 0.5        | 0.8             |
| CT           | 80 (0.42)                         | 327 (0.44)                   | 0.895 (0.633–1.265) | 0.6          |            |                 |
| CC           | 16 (0.08)                         | 66 (0.09)                    | 0.887 (0.458–1.636) | 0.8          |            |                 |
| CT+CC        | 96 (0.50)                         | 393 (0.53)                   | 0.894 (0.643–1.243) | 0.5          |            |                 |
| MAF          | 0.29                              | 0.31                         | 0.924 (0.715–1.187) | 0.6          |            |                 |
| VDR rs2228570| n=189                             | n=745                        |                    |              |            |                 |
| CC           | 62 (0.33)                         | 274 (0.37)                   | Reference          | –            | 0.3        | 0.5             |
| CT           | 92 (0.49)                         | 351 (0.47)                   | 1.158 (0.798–1.689) | 0.5          |            |                 |
| TT           | 35 (0.18)                         | 120 (0.16)                   | 1.289 (0.782–2.101) | 0.3          |            |                 |
| CT+TT        | 127 (0.67)                        | 471 (0.63)                   | 1.192 (0.840–1.702) | 0.4          |            |                 |
| MAF          | 0.43                              | 0.40                         | 1.141 (0.901–1.443) | 0.3          |            |                 |
| VDR rs1544410| n=192                             | n=743                        |                    |              |            |                 |
| GG           | 73 (0.38)                         | 301 (0.41)                   | Reference          | –            | 0.4        | 0.7             |
| AG           | 90 (0.47)                         | 343 (0.46)                   | 1.082 (0.755–1.553) | 0.7          |            |                 |
| AA           | 29 (0.15)                         | 99 (0.13)                    | 1.208 (0.714–2.006) | 0.5          |            |                 |
| AG+AA        | 119 (0.62)                        | 442 (0.59)                   | 1.110 (0.792–1.563) | 0.6          |            |                 |
| MAF          | 0.39                              | 0.36                         | 1.095 (0.863–1.388) | 0.5          |            |                 |
Table 2 continued. Comparison of the distribution of VD pathway gene polymorphisms between ChGN patients showing GFR category 1–3 and healthy subjects.

| Genotype       | ChGN GFR category 1–3 (frequency) | Healthy subjects (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | P_genotyping |
|----------------|-----------------------------------|-------------------------------|--------------------|--------------|---------|-------------|
| RXRA rs10776909| n=195                             | n=751                         |                    |              |         |             |
| CC             | 122 (0.63)                        | 498 (0.66)                    | Reference          | –            | 0.3     | 0.6         |
| CT             | 65 (0.33)                         | 227 (0.30)                    | 1.169 (0.818–1.661)| 0.4          |         |             |
| TT             | 8 (0.04)                          | 26 (0.04)                     | 1.256 (0.479–2.945)| 0.7          |         |             |
| CT+TT          | 73 (0.37)                         | 253 (0.34)                    | 1.178 (0.836–1.652)| 0.4          |         |             |
| MAF            | 0.21                              | 0.19                          | 1.149 (0.859–1.526)| 0.4          |         |             |
| RXRA rs10881578| n=194                             | n=750                         |                    |              |         |             |
| AA             | 89 (0.46)                         | 382 (0.51)                    | Reference          | –            | 0.3     | 0.4         |
| AG             | 85 (0.44)                         | 294 (0.39)                    | 1.241 (0.876–1.757)| 0.2          |         |             |
| GG             | 20 (0.10)                         | 74 (0.10)                     | 1.160 (0.636–2.043)| 0.7          |         |             |
| AG+GG          | 105 (0.54)                        | 368 (0.49)                    | 1.225 (0.881–1.703)| 0.2          |         |             |
| MAF            | 0.32                              | 0.29                          | 1.138 (0.887–1.455)| 0.3          |         |             |
| RXRA rs749759  | n=188                             | n=728                         |                    |              |         |             |
| GG             | 103 (0.55)                        | 432 (0.59)                    | Reference          | –            | 0.3     | 0.5         |
| AG             | 71 (0.38)                         | 244 (0.34)                    | 1.220 (0.854–1.738)| 0.3          |         |             |
| AA             | 14 (0.07)                         | 52 (0.07)                     | 1.129 (0.556–2.166)| 0.8          |         |             |
| AG+AA          | 85 (0.35)                         | 296 (0.41)                    | 1.204 (0.860–1.684)| 0.3          |         |             |
| MAF            | 0.26                              | 0.24                          | 1.138 (0.868–1.484)| 0.4          |         |             |

ChGN – chronic glomerulonephritis; GFR – glomerular filtration rate; MAF – minor allele frequency; VD – vitamin D.

In GFR category 5D ChGN patients, frequencies of RXRA rs10776909 MAF (25% vs. 19%), CT (42% vs. 30%), and CT+TT (46% vs. 34%) were higher compared to frequencies of respective polymorphic variant in controls (P_trend=0.004, P_genotyping=0.008) (Table 5). After stratification for potentially confounding factors such as sex or age, all significant differences were maintained (Supplementary material online, Table 3).

When frequencies of RXRA rs10776909 genotypes and allele of all ChGN patients (n=373) were compared to those of controls, higher frequency of allele T in ChGN subjects was demonstrated (Table 6). Significance was slightly weaker after adjustment for age and sex, but maintained (Supplementary material online, Table 4).

Discussion

Despite clinical and experimental evidence indicating associations of 1,25(OH)₂D₃, VD binding protein and VDR with glomerular injury [1–5], associations of nucleotide variants of GC and VDR with ChGN were not found in the current study, either in early or in late disease stages. The only polymorphism significantly associated with ChGN was that of RXRA rs10776909. ChGN subjects with GFR category 1–3 and 5D being analyzed as a whole group showed higher RXRA rs10776909 allele T frequency than healthy subjects did.

VDR forms a heterodimer with RXR to regulate target gene transcription [10]. RXRA encodes RXR alpha, but the function of the protein product of this gene is still not completely understood. Ovsyannikova et al. [11] studied immune responses to measles vaccine, reporting a significant association in whites between lower measles-specific IFN-γ Elispot responses and...
Table 3. Comparison of the distribution of VD pathway gene polymorphisms between ChGN patients showing GFR category 1–3 and GFR category 5D.

| Genotype | ChGN GFR category 5D (frequency) | ChGN GFR category 1–3 (frequency) | Odds ratio (95%CI) | Two-tailed P | \( P_{\text{trend}} \) | \( P_{\text{genotyping}} \) |
|----------|----------------------------------|----------------------------------|--------------------|--------------|-------------------|-------------------|
| **GC rs2298849** | | | | | | |
| TT       | 119 (0.67) | 123 (0.64) | Reference | – | 0.6 | 0.7 |
| CT       | 52 (0.29) | 64 (0.33) | 0.840 (0.525–1.341) | 0.5 | | |
| CC       | 6 (0.03) | 6 (0.03) | 1.034 (0.268–3.984) | 1.0 | | |
| CT+CC    | 58 (0.33) | 70 (0.36) | 0.856 (0.544–1.346) | 0.6 | | |
| MAF      | 0.18 | 0.20 | 1.138 (0.868–1.484) | 0.4 | | |
| **GC rs7041** | | | | | | |
| GG       | 50 (0.29) | 63 (0.33) | Reference | – | 0.7 | 0.7 |
| GT       | 88 (0.51) | 90 (0.46) | 1.232 (0.747–2.035) | 0.5 | | |
| TT       | 34 (0.20) | 40 (0.21) | 1.071 (0.569–2.011) | 0.9 | | |
| GT+TT    | 122 (0.71) | 130 (0.67) | 1.182 (0.739–1.896) | 0.5 | | |
| MAF      | 0.45 | 0.44 | 1.054 (0.779–1.427) | 0.8 | | |
| **GC rs1155563** | | | | | | |
| TT       | 81 (0.45) | 97 (0.50) | Reference | – | 0.2 | 0.3 |
| CT       | 74 (0.42) | 80 (0.42) | 1.108 (0.702–1.747) | 0.7 | | |
| CC       | 23 (0.13) | 16 (0.08) | 1.721 (0.807–3.732) | 0.2 | | |
| CT+CC    | 97 (0.54) | 96 (0.50) | 1.210 (0.788–1.858) | 0.4 | | |
| MAF      | 0.34 | 0.29 | 1.244 (0.901–1.718) | 0.2 | | |
| **VDR rs2228570** | | | | | | |
| CC       | 46 (0.26) | 62 (0.33) | Reference | – | 0.5 | 0.3 |
| CT       | 98 (0.57) | 92 (0.49) | 1.436 (0.868–2.379) | 0.2 | | |
| TT       | 29 (0.17) | 35 (0.18) | 1.117 (0.571–2.179) | 0.8 | | |
| CT+TT    | 127 (0.73) | 127 (0.67) | 1.348 (0.836–2.181) | 0.2 | | |
| MAF      | 0.45 | 0.43 | 1.095 (0.807–1.485) | 0.6 | | |
| **VDR rs1544410** | | | | | | |
| GC       | 67 (0.38) | 73 (0.38) | Reference | – | 0.9 | 1.0 |
| AG       | 82 (0.47) | 90 (0.47) | 0.993 (0.620–1.591) | 1.0 | | |
| AA       | 26 (0.15) | 29 (0.15) | 0.977 (0.498–1.912) | 1.0 | | |
| AG+AA    | 108 (0.62) | 119 (0.62) | 0.989 (0.635–1.542) | 1.0 | | |
| MAF      | 0.38 | 0.39 | 0.989 (0.726–1.347) | 1.0 | | |

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Table 3 continued. Comparison of the distribution of VD pathway gene polymorphisms between ChGN patients showing GFR category 1–3 and GFR category 5D.

| Genotype | ChGN GFR category 5D (frequency) | ChGN GFR category 1–3 (frequency) | Odds ratio (95%CI) | Two-tailed P | P<sub>trend</sub> | P<sub>genotyping</sub> |
|----------|---------------------------------|---------------------------------|-------------------|--------------|----------------|-------------------|
| RXRA rs10776909 | n=178                           | n=195                           |                   |              |                |                   |
| CC       | 96 (0.54)                       | 122 (0.63)                      | Reference         | –            | 0.1            | 0.2               |
| CT       | 74 (0.42)                       | 65 (0.33)                       | 1.447 (0.923–2.269) | 0.1         |                |                   |
| TT       | 8 (0.04)                        | 8 (0.04)                        | 1.271 (0.399–4.037) | 0.8         |                |                   |
| CT+TT    | 82 (0.46)                       | 73 (0.37)                       | 1.428 (0.924–2.205) | 0.1         |                |                   |
| MAF      | 0.25                            | 0.21                            | 1.291 (0.904–1.845) | 0.2         |                |                   |
| RXRA rs10881578 | n=178                           | n=194                           |                   |              |                |                   |
| AA       | 76 (0.43)                       | 89 (0.46)                       | Reference         | –            | 0.7            | 0.7               |
| AG       | 85 (0.48)                       | 85 (0.44)                       | 1.171 (0.745–1.840) | 0.5         |                |                   |
| GG       | 17 (0.09)                       | 20 (0.10)                       | 0.995 (0.454–2.162) | 1.0         |                |                   |
| AG+GG    | 102 (0.57)                      | 105 (0.54)                      | 1.138 (0.740–1.750) | 0.6         |                |                   |
| MAF      | 0.33                            | 0.32                            | 1.056 (0.768–1.452) | 0.8         |                |                   |
| RXRA rs749759 | n=177                           | n=188                           |                   |              |                |                   |
| GG       | 93 (0.53)                       | 103 (0.55)                      | Reference         | –            | 0.7            | 0.9               |
| AG       | 71 (0.40)                       | 71 (0.38)                       | 1.108 (0.702–1.746) | 0.7         |                |                   |
| AA       | 13 (0.07)                       | 14 (0.07)                       | 1.028 (0.421–2.496) | 1.0         |                |                   |
| AG+AA    | 84 (0.47)                       | 85 (0.45)                       | 1.094 (0.710–1.687) | 0.7         |                |                   |
| MAF      | 0.27                            | 0.26                            | 1.056 (0.751–1.485) | 0.8         |                |                   |

ChGN – chronic glomerulonephritis; GFR – glomerular filtration rate; MAF – minor allele frequency; VD – vitamin D.

RXRA haplotype, including major allele of rs10776909 among 7 other RXRA alleles. We have shown that RXRA polymorphic variants (rs10776909, rs10881578, rs749759) were not involved in determining response to hepatitis B vaccination in dialysis patients [12].

Retinoid acid receptors and RXRs with isoforms alpha, beta, and gamma are expressed in kidneys and codetermine the final number of glomeruli in rats [13]. In the studies by Schaier et al. [6], gene expression of RXR alpha was markedly higher in glomeruli of nephritic than non-nephritic rats. AGN 194204, having a high specificity to RXRs, decreased mesangial cell proliferation, the glomerular cell count per glomerular section, mesangial matrix expansion, the glomerulosclerosis index, the tubulointerstitial area, the interstitial cell count, the number of glomerular monocytes/macrophages (ED-1+ cells), expression for the gene for transforming growth factor beta in glomeruli, and expression of RXR alpha. These changes were accompanied in nephritic rats by decreased blood pressure and albuminuria, but creatinine clearance remained unchanged. Therefore, RXR-specific agonist reduced renal injury in established ChGN in rats [6].

Distribution of nephrotic syndrome in GFR 1–3 patients selected according to RXRA rs10776909 genotypes did not differ significantly; however, this finding was influenced by the small number of TT homozygotes among tested individuals. Nephrotic syndrome was found to be associated with glomerular hyperfiltration (GFR >150 ml/min/1.73 m<sup>2</sup> in this study) in 35.5% of subjects, with minimal change in disease [14]. The significantly lower serum creatinine concentrations in TT genotype bearers suggest that this genotype may be associated with glomerular hyperfiltration occurring in some patients with nephrotic syndrome. Glomerular hyperfiltration seems to be...
Table 4. Selected clinical/laboratory features of GFR category 1–3 ChGN patients in relation to RXRA rs10776909 polymorphic variant.

| Parameter                        | RXRA rs10776909 | Model of inheritance | Odds ratio (95% CI) | P value |
|----------------------------------|-----------------|----------------------|---------------------|---------|
| Nephrotic syndrome               |                 | CC vs. CT + TT       | 1.294 (0.695–2.416) | 0.5     |
|                                  |                 | CC + CT vs. TT       | 3.032 (0.522–31.30) | 0.3     |
|                                  |                 | CC vs. TT            | 3.203 (0.541–33.39) | 0.3     |
| Creatinine concentration, mg/dl  |                 | CC vs. CT + TT       | 1.0*                | 0.01*   |
|                                  |                 | CC + CT vs. TT       | 0.01* ***           |         |
|                                  |                 | CC vs. TT            | 0.02* ****          |         |
| eGFR, ml/min/1.73 m²             |                 | CC vs. CT + TT       | 0.5*                |         |
|                                  |                 | CC + CT vs. TT       | 0.1*                |         |
|                                  |                 | CC vs. TT            | 0.1**               |         |

Data are presented as median (minimum–maximum), or number (percentage). * Mann-Whitney test; ** Cochran Cox test; *** significant after the Bonferroni correction (P<0.017); **** nonsignificant after the Bonferroni correction (P>0.017). Conversion factor to SI units for creatinine is: 1 mg/dl = 88.4 μmol/l. ChGN – chronic glomerulonephritis; GFR – glomerular filtration rate.

associated with a poorer prognosis [14]. Therefore, it should not be a surprise that frequency of allele T is higher in ChGN patients with severely deteriorated renal function. However, a prospective study showing a clinical course of ChGN in patients differing in RXRA rs10776909 genotypes could be reasonable to confirm (or exclude) the role of allele T in progression of the disease.

Glomerular hyperfiltration occurs in several clinical conditions [8,15–20], also in type 1 diabetes mellitus (DM) [19] as well as type 2 DM [8,20]. It is seen early in the course of DM and persists until the time macroalbuminuria appears. Glomerular hyperfiltration in DM did not predict worsening albuminuria or declining GFR during 4-year follow-up [20], but it is associated with a poor prognosis for development of diabetic kidney disease [9]. In our previous study, there were no significant differences in a frequency distribution of RXRA rs10776909 polymorphic variants between type 2 DM nephropathy patients in GFR category 5D and controls [21]. This indicates that RXRA rs10776909 polymorphism is specifically related to severity of ChGN but not to end-stage type 2 diabetic nephropathy.

Individual clinical presentation and survival rate in primary ChGN markedly depend on histological changes in glomeruli [22]. The weak point of our study is the relatively small number of ChGN patients, and a lack of renal biopsates in most patients with GFR category 5D. Our findings may lead to further exploration of the VD-related genetic basis of specific types of ChGN.
Table 5. Comparison of the distribution of VD pathway gene polymorphisms between ChGN patients showing GFR category 5D and healthy subjects.

| Genotype     | ChGN GFR category 5D (frequency) | Healthy subjects (frequency) | Odds ratio (95%CI) | Two-tailed P | P trend | P genotyping |
|--------------|----------------------------------|------------------------------|--------------------|---------------|---------|--------------|
| GC rs2298849 |                                  |                              |                    |               |         |              |
| TT           | 119 (0.67)                       | 461 (0.62)                   | Reference          | –             | 0.2     | 0.4          |
| CT           | 52 (0.29)                        | 257 (0.34)                   | 0.784 (0.536–1.137) | 0.2           |         |              |
| CC           | 6 (0.03)                         | 30 (0.04)                    | 0.775 (0.258–1.951) | 0.8           |         |              |
| CT+CC        | 58 (0.33)                       | 287 (0.38)                   | 0.783 (0.543–1.120) | 0.2           |         |              |
| MAF          | 0.18                             | 0.21                         | 0.821 (0.599–1.112) | 0.2           |         |              |
| GC rs7041    |                                  |                              |                    |               |         |              |
| GG           | 50 (0.29)                        | 244 (0.34)                   | Reference          | –             | 0.2     | 0.4          |
| GT           | 88 (0.51)                        | 362 (0.50)                   | 1.186 (0.797–1.780) | 0.4           |         |              |
| TT           | 34 (0.20)                        | 122 (0.17)                   | 1.360 (0.807–2.270) | 0.3           |         |              |
| GT+TT        | 122 (0.71)                       | 484 (0.66)                   | 1.230 (0.845–1.808) | 0.3           |         |              |
| MAF          | 0.45                             | 0.42                         | 1.164 (0.912–1.484) | 0.2           |         |              |
| GC rs1155563 |                                  |                              |                    |               |         |              |
| TT           | 81 (0.45)                        | 355 (0.47)                   | Reference          | –             | 0.3     | 0.2          |
| CT           | 74 (0.42)                        | 327 (0.44)                   | 0.992 (0.688–1.428) | 1.0           |         |              |
| CC           | 23 (0.13)                        | 66 (0.09)                    | 1.527 (0.853–2.662) | 0.2           |         |              |
| CT+CC        | 97 (0.54)                        | 393 (0.53)                   | 1.082 (0.769–1.524) | 0.7           |         |              |
| MAF          | 0.34                             | 0.31                         | 1.149 (0.890–1.478) | 0.3           |         |              |
| VDR rs2228570|                                  |                              |                    |               |         |              |
| CC           | 46 (0.26)                        | 274 (0.37)                   | Reference          | –             | 0.06    | 0.03         |
| CT           | 98 (0.57)                        | 351 (0.47)                   | 1.663 (1.116–2.501) | 0.01          |         |              |
| TT           | 29 (0.17)                        | 120 (0.16)                   | 1.439 (0.829–2.467) | 0.2           |         |              |
| CT+TT        | 127 (0.73)                       | 471 (0.63)                   | 1.606 (1.098–2.377) | 0.01          |         |              |
| MAF          | 0.45                             | 0.40                         | 1.249 (0.979–1.592) | 0.07          |         |              |
| VDR rs1544410|                                  |                              |                    |               |         |              |
| GG           | 67 (0.38)                        | 301 (0.41)                   | Reference          | –             | 0.5     | 0.8          |
| AG           | 82 (0.47)                        | 343 (0.46)                   | 1.074 (0.740–1.563) | 0.8           |         |              |
| AA           | 26 (0.15)                        | 99 (0.13)                    | 1.180 (0.680–2.002) | 0.6           |         |              |
| AG+AA        | 108 (0.62)                       | 442 (0.59)                   | 1.098 (0.773–1.566) | 0.7           |         |              |
| MAF          | 0.38                             | 0.36                         | 1.084 (0.845–1.386) | 0.6           |         |              |
Table 5 continued. Comparison of the distribution of VD pathway gene polymorphisms between ChGN patients showing GFR category 5D and healthy subjects.

| Genotype | ChGN GFR category 5D (frequency) | Healthy subjects (frequency) | Odds ratio (95%CI) | Two-tailed P | P trend | P genotyping |
|----------|----------------------------------|-----------------------------|--------------------|--------------|---------|--------------|
| RXRA rs10776909 | n=178 | n=751 | | | |
| CC | 96 (0.54) | 498 (0.66) | Reference | — | 0.004 | 0.008 |
| CT | 74 (0.42) | 227 (0.30) | 1.691 (1.182–2.411) | 0.004 |
| TT | 8 (0.04) | 26 (0.04) | 1.596 (0.605–3.766) | 0.4 |
| CT+TT | 82 (0.46) | 253 (0.34) | 1.681 (1.189–2.372) | 0.003 |
| MAF | 0.25 | 0.19 | 1.483 (1.116–1.960) | 0.006 |
| RXRA rs10881578 | n=178 | n=750 | | | |
| AA | 76 (0.43) | 382 (0.51) | Reference | — | 0.2 | 0.1 |
| AG | 85 (0.48) | 294 (0.39) | 1.453 (1.014–2.083) | 0.04 |
| GG | 17 (0.09) | 74 (0.10) | 1.155 (0.604–2.112) | 0.7 |
| AG+GG | 102 (0.57) | 368 (0.49) | 1.393 (0.989–1.967) | 0.04 |
| MAF | 0.33 | 0.29 | 1.202 (0.930–1.547) | 0.2 |
| RXRA rs749759 | n=177 | n=728 | | | |
| GG | 93 (0.53) | 432 (0.59) | Reference | — | 0.2 | 0.2 |
| AG | 71 (0.40) | 244 (0.34) | 1.352 (0.940–1.938) | 0.1 |
| AA | 13 (0.07) | 52 (0.07) | 1.161 (0.557–2.272) | 0.8 |
| AG+AA | 84 (0.47) | 296 (0.41) | 1.319 (0.934–1.858) | 0.1 |
| MAF | 0.27 | 0.24 | 1.202 (0.913–1.573) | 0.2 |

ChGN – chronic glomerulonephritis; GFR – glomerular filtration rate; MAF – minor allele frequency; VD – vitamin D. Genotype distributions in all data sets are consistent with Hardy-Weinberg equilibrium.

Table 6. Comparison of the distribution of RXRA rs10776909 polymorphism between all ChGN patients and healthy subjects.

| Genotype | All ChGN patients (frequency) | Healthy subjects (frequency) | Odds ratio (95%CI) | Two-tailed P | P trend | P genotyping |
|----------|-------------------------------|-----------------------------|--------------------|--------------|---------|--------------|
| RXRA rs10776909 | n=373 | n=751 | | | |
| CC | 218 (0.58) | 498 (0.66) | Reference | — | 0.01 | 0.04 |
| CT | 139 (0.37) | 227 (0.30) | 1.399 (1.064–1.837) | 0.02 |
| TT | 16 (0.04) | 26 (0.04) | 1.406 (0.689–2.783) | 0.4 |
| CT+TT | 155 (0.42) | 253 (0.34) | 1.400 (1.074–1.821) | 0.01 |
| MAF | 0.23 | 0.19 | 1.304 (1.045–1.624) | 0.02 |
| P for HWE | 0.573 | 0.999 | | | |

ChGN – chronic glomerulonephritis; HWE – Hardy-Weinberg equilibrium; MAF – minor allele frequency.
Conclusions

1. The current study for the first time shows that RXRA rs10776909 allele T is specifically involved in pathogenesis of ChGN and may worsen the clinical course of this disease.

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