Calcium-sensing receptor gene (CASR) polymorphisms and CASR transcript level concerning dyslipidemia in hemodialysis patients: a cross-sectional study

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Abstract

Background: There is scarce data on CASR associations with dyslipidemia. We investigated in hemodialysis (HD) patients whether CASR single nucleotide polymorphisms (SNPs) rs7652589 and rs1801725 have associations with dyslipidemia and show epistatic interactions with SNPs of the energy homeostasis-associated gene (ENHO), retinoid X receptor α gene (RXRA), and liver X receptor α gene (LXRA).

Methods: The study included 1208 HD subjects. For diagnosis of dyslipidemia, both K/DOQI criteria and atherogenic index ≥3.8 were used. CASR rs1801725 was genotyped by TaqMan SNP Genotyping Assay, other SNPs – by high-resolution melting curve analysis or polymerase chain reaction-restriction fragment length polymorphism, as appropriate. Relative transcript levels of CASR, ENHO, RXRA, and LXRA were measured in peripheral blood mononuclear cells. The occurrence of dyslipidemic phenotypes concerning tested polymorphisms was compared using models of inheritance. Haplotypes were estimated using the Haploview 4.2 software. Epistatic interactions between tested SNPs were analyzed using the logistic regression and epistasis option in the PLINK software.

Results: Rs7652589 indicated a greater probability of atherogenic dyslipidemia in the dominant inheritance model (OR 1.4, 95%CI 1.0–2.0, P = 0.026), principally because of increased triglyceride (TG) levels. The rs1801725 variant allele was associated with a decreased probability of dyslipidemia characterized by non-HDL-cholesterol ≥130 mg/dL and TG ≥200 mg/dL (OR 0.6, 0.4–0.9, P = 0.012). There were no epistatic interactions between CASR and RXRA, LXRA, and ENHO regarding dyslipidemia. Both rs7652589 and rs1801725 SNPs were not in linkage disequilibrium (D’ = 0.091, r2 = 0.003 for the entire HD group) and their haplotypes did not correlate with dyslipidemia. Relative CASR transcript was lower at a borderline significance level in patients harboring the rs1801725 variant allele compared with homozygotes of the major allele (0.20, 0.06–7.80 vs. 0.43, 0.04–5.06, P = 0.058). CASR transcript correlated positively with RXRA transcript (adjusted P = 0.001), LXRA transcript (adjusted P = 0.0009), ENHO transcript (borderline significance, adjusted P = 0.055), dry body weight (adjusted P = 0.035), and renal replacement therapy duration (adjusted P = 0.013).

Conclusions: CASR polymorphisms (rs7652589, rs1801725) are associated with dyslipidemia in HD patients. CASR correlates with RXRA, LXRA, and ENHO at the transcript level. Further investigations may elucidate whether other CASR SNPs contribute to associations shown in this study.

Keywords: CASR, Dyslipidemia, ENHO, Hemodialysis, LXRA, RXRA, Transcript level
Background
Patients requiring hemodialysis (HD) treatment show multiple metabolic disturbances, including dyslipidemias. Lipid alterations, among them atherogenic dyslipidemia, contribute to initiation and progression of coronary artery disease (CAD), myocardial infarction (MI), and premature death. In end-stage renal disease patients, dyslipidemias were defined by the National Kidney Foundation/Kidney Disease Outcomes Quality Initiative (K/DOQI) as serum low-density lipoprotein (LDL)-cholesterol ≥100 mg/dL or simultaneously occurring non-high density lipoprotein (non-HDL)-cholesterol ≥130 mg/dL and triglycerides (TG) ≥200 mg/dL [1]. Atherogenic dyslipidemia is frequently referred to as the atherogenic index calculated as TG to high-density lipoprotein (HDL)-cholesterol ratio which is equal to or over 3.8 [2]. Although 79.8% of HD patients are reported to have abnormal serum levels [1], the etiology of dyslipidemias in this group of patients is still insufficiently elucidated. In uremic subjects, atherosclerotic plaques and vascular calcifications are accompanied by mineral disorders, increased calcium-phosphorus product, and advanced secondary hyperparathyroidism [3].

Single nucleotide polymorphisms (SNPs) of calcium-sensing receptor gene (CASR) are predominantly associated with phenotypes of primary [4] and secondary hyperparathyroidism [5–8], and with idiopathic calcium nephrolithiasis [9, 10]. However, CASR expression is shown not only in parathyroid glands [11] and kidney tubules [10], but also in vascular smooth muscle cells [12], adipocytes and their progenitor cells [13, 14], human omental tissue [14], and hepatocytes [14, 15]. It is suggested that livers from obese patients may express higher levels of CASR transcripts [14]. Reduced CASR mRNA levels were attributed to variant alleles of CASR rs7652589 [7, 16] and rs1501899 [16]. An increase of Ca²⁺ in cytosol due to activation of the calcium-sensing receptor (CaSR) may influence adipogenesis and accumulation of TG in adipocytes [13]. It is suggested that CaSR has antilipolytic effect in human adipocytes [13]. On the other hand, CaSR activation by calcimimetic cinacalcet decreased adipocyte TG content by 20% [14]. Antilipolytic effect of calcimetics was attributed to the allelic variant of CASR polymorphism rs1042636 (Arg990Gly) [17]. Additionally, up-regulation of CaSR was found to activate the peroxisome proliferator-activated receptor α (PPARα) which is a transcription factor involved in the regulation of adipogenesis [18]. PPARs heterodimerize with retinoid X receptors (RXR) [19]. RXRα and liver X receptor α (LXRA) form the functional heterodimer LXRA-RXRα [20]. Up-regulation of LXRA diminishes the liver expression of the energy homeostasis-associated gene (ENHO) [21]. ENHO, RXRA, and LXRA SNPs were associated separately or jointly with dyslipidemia, MI, and survival in HD patients [22].

Several CASR SNPs were studied for their relationships with serum total cholesterol concentration, but results were negative, at least in renal transplant recipients [23]. The CASR R990G (rs1042636) polymorphism was associated with increased risk of hypertriglyceridemia in the Chinese population, especially in obese individuals, but other two nonsynonymous CASR coding region SNPs (A986S rs1801725, Q1011E rs1801726) were distributed similarly in hypertriglyceridemic and non-hypertriglyceridemic subjects [24]. In Caucasians, the variant allele in CASR rs1801725 (but not homozygosity of the variant allele) was reported to be an independent predictor of CAD, MI, and cardiovascular mortality [25].

As rs7652589 was previously associated with secondary hyperparathyroidism in HD patients [5–8] and rs1801725 was reported as related to CAD, MI, and cardiovascular mortality in Caucasians [25], we have chosen these two SNPs for searching their associations with dyslipidemia in HD subjects. Dyslipidemia may be a factor which together with calcium disturbances contributes to atherogenic cardiac disease and cardiovascular mortality. HD patients, presenting both dyslipidemia and secondary hyperparathyroidism, seem to be a unique group to study CASR polymorphisms that are associated with calcium disorders and possibly also with dyslipidemia. Both CASR SNPs (rs7652589 located 13 kbp upstream from the TATA box of promoter 1 and a missense variant rs1801725 located in exon 7 on chromosome 3) are not in linkage disequilibrium (LD), so their associations are not obvious and therefore worth to investigate.

The aim of our study was to investigate whether CASR SNPs (rs1801725, rs7652589) are associated with dyslipidemia in HD patients, or whether there is any interaction between CASR and other genes known as associated with lipogenesis, like ENHO, retinoid X receptor α gene (RXRA), or liver X receptor α gene (LXRA). CASR, ENHO, RXRA, and LXRA transcripts were also tested for correlations.

Methods
Patients
To be enrolled in the study, HD patients had to fulfill the following criteria:

1. not to show secondary causes of dyslipidemia (hypothyroidism, alcohol abuse, medication with anticonvulsants, corticosteroid therapy) and cachectic conditions causing decreases in serum lipids (neoplasms, enteropathies, liver cirrhosis),
2. not to receive treatment with cinacalcet at least for
   6 months before determination of serum lipid
   profile,
3. to have a serum lipid profile determined in stable
   general condition.

HD patients were qualified as candidates for this study
independently on treatment with lipid-lowering medica-
tions. However, to be included as not receiving lipid-
lowering therapy, the patients had to be free from lipid-
lowering medicines for at least 6 months before to deter-
mination of serum lipid profile used in this study. To be
included as receiving lipid-lowering therapy, the patients
had to undergo lipid-lowering medications for at least 6
months before the determination of serum lipid concen-
trations used in this study. Patients, who did not fulfill
these criteria, were excluded.

HD subjects were diagnosed as having dyslipidemia by
the use of the K/DOQI criteria [1] and also by applying
the atherogenic index [2].

All the study participants (n = 1208) were Caucasians
of Polish origin.

Laboratory examinations
In all studied HD individuals, blood samples were taken
before the midweek HD session for CASR, ENHO, RXRA,
and LXRA polymorphisms, serum lipids (total cholesterol
- TC, HDL-cholesterol, TG), and laboratory parameters
routinely tested in HD subjects.

Serum lipids which were determined using enzymatic
colorimetric tests (Roch e Diagnostics, Mannheim,
Germany). The LDL-cholesterol level was computed by
the Friedewald equation [26]. If serum TG levels equal
to or exceeding 400 mg/dL, LDL-cholesterol was deter-
mined in 112 HD patients. Due to a risk of complica-
tions used in this study. Patients, who did not fulfill
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mined directly (BioSystems S.A., Reagents and Instru-
ments, Barcelona, Spain). Non-HDL cholesterol was
calculated as the TC minus HDL-cholesterol.

Genotyping
Tested SNPs in CASR (rs7652589, rs1801725), ENHO
(rs2281997, rs72735260), RXRA (rs749759, rs10776909,
rs10881578), and LXRA (rs2279238, rs7120118, rs11039155)
were characterized using public databases including the
NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/
projects/SNP/) and the 1000 Genomes Browser (http://
browser.1000genomes.org/index.html). SNPs were selected
based on variant (minor) allele frequency (MAF) exceeding
5% in the Caucasian population and gene LD patterns.
Characteristics of the tested polymorphisms are displayed in
Additional file 1: Table S1. Genomic locations of CASR
rs7652589 (A < G) and rs1801725 (G > T) are shown in
Additional file 1: Figure S1.

DNA for genotype analysis was extracted from blood
lymphocytes by the salt-out extraction method. CASR
rs7652589, RXRA SNPs, and ENHO SNPs were geno-
typed as previously described [7, 27]. Analysis of CASR
rs1801725 variant was performed using predesigned
TaqMan SNP Genotyping Assay according to the manu-
facturer’s instructions provided by Applied Biosystems
(Applied Biosystems, Foster City, CA). Genotyping of
LXRA SNPs performed using high-resolution melting
curve (HRM) analysis on the Light Cycler 480 system
(Roche Diagnostics, Germany). In brief, DNA fragments
amplified with the use of specific primers were subjected
to HRM with 0.1 °C increments in temperatures ranging
from 70 to 92 °C. For quality control, approximately 20% of
the randomly chosen samples were re-genotyped. Samples with ambiguous results were excluded from fur-
ther statistical analyses.

Genotyping was performed using encoded blood
samples.

CASR rs7652589 and CASR rs1801725 genotypes
were obtained in 1139 and 1159 patients, respectively.
ENHO rs2281997 was successfully genotyped in 1182
patients, ENHO rs72735260 – in 1183 subjects. Geno-
typing for RXRA SNPs was performed with excess in 1196
patients for rs749759, 1199 patients for rs10776909, and in 1200 for rs10881578. LXRA SNPs
(rs2279238, rs7120118, rs11039155) were successfully ge-
notyped in 1186, 1188, and 1189 patients, respectively.

Distributions of tested polymorphisms were in con-
cordance with the Hardy-Weinberg equilibrium (HWE).

Reverse transcription-quantitative polymerase chain
reaction (qPCR) analysis
CASR, ENHO, RXRA, and LXRA transcripts were deter-
mined in 112 HD patients. Due to a risk of complica-
tions, mainly bleeding during the collection of tissue
material in HD patients, the only available cell material
for the transcript determination was that composed of
peripheral blood mononuclear cells (PBMCs). They were
isolated by density-gradient centrifugation using the
Histopaque (Sigma-Aldrich, Missouri, United States).
Cells were washed in phosphate-buffered saline, and
total RNA isolation was performed according to the
method of Chomczynski and Sacchi [28]. The concen-
tration and integrity of the isolated RNA were assessed
by spectrophotometric quantification and nondenatur-
ing electrophoresis on a 2% agarose gel. RNA samples
were treated with Ambion DNase I (Thermo Fisher
Scientific, Inc., Waltham, MA, USA) and 1.5 μg of
RNA was reverse-transcribed into complementary
DNA (cDNA) using an M-MLV Reverse Transcriptase
(Thermo Fisher Scientific, Inc., Waltham, MA, USA)
according to the manufacturer’s protocol. Quantitative
analyses were performed using a LightCycler® 480
Real-Time PCR system (Roche Diagnostics GmbH,
Mannheim, Germany). The transcripts of target genes
were quantified by the relative quantification method using a calibrator, as is described in the Relative Quantification Manual (Roche Diagnostics GmbH, Mannheim, Germany) [29]. The calibrator contained the cDNA mix from all analyzed samples. Each qPCR mix contained 1 μl of cDNA, 9 μl LightCycler 480 SYBR Green I Master Mix (Roche Diagnostics GmbH) and 0.25 μM of the corresponding primers. Primers sequence for CASR was used as previously [7] whereas all other primers were newly designed using OLGOS Primer Analysis Software (version 5.0; Molecular Biology Insights, Inc., Colorado Springs, CO, USA) in our laboratory. Sequences for these primers were used as follow: ENHO, F: CAGGCTCAACTCAGCTCAG and R: GAGGAG GCTGTGCTGTCTGC; LXRA, F: CGAGGTGATGCTTC TGGAGA and R: CCTGGAGAACCTCGAAGATGG; RXRA F: TTCCTCTTTAAACCTGACTCC and R: AGAG CTTAGCGAACCTTCC. The transcript amounts were calculated as the ratio between the quantity of target transcript in a sample and target transcript in the calibrator. The occurrence of dyslipidemic phenotypes concerning tested polymorphisms was compared using models of inheritance (dominant, recessive, additive) in four HD groups:

1. composed of subjects dyslipidemic by K/DOQI criteria, atherogenic index, or both among patients not receiving lipid-lowering medication together with all patients treated with antilipemic medicines,
2. with an exclusion of patients in whom dyslipidemia was abolished by antilipemic medicines (patients who were dyslipidemic on antilipemic treatment were assumed to have the same type of dyslipidemia as before initiation of antilipemic medication),
3. comprised of patients not receiving antilipemic medicines,
4. composed of patients receiving antilipemic medication.

All four groups were compared with subjects free of dyslipidemia by both criteria not receiving antilipemic medication. Patients showing dyslipidemia by K/DOQI rules [1] or by the atherogenic index [2] were compared with patients without dyslipidemia by a respective criterion. Among patients dyslipidemic by K/DOQI criteria [1], the group showing plasma LDL-cholesterol concentration ≥ 100 mg/dL and the group presenting non-HDL-cholesterol ≥130 mg/dL and TG ≥ 200 mg/dL were also analyzed separately.

For selected categorical variables, Odds ratio (OR) and 95% confidence intervals (CIs) for OR were calculated. Chi-square test or Fisher’s test was used for statistical evaluation of OR. All probabilities were two-tailed, and P-value below 0.05 was considered significant.

To determine the associations of selected SNPs with appropriate phenotypes among other relevant patient characteristics, we used the primary logistic regression models with subsequent stepwise logistic regression and backward elimination for selection of significant variables among other possible determinants of tested dyslipidemic phenotypes.

Abovementioned statistical analyses were performed using R software version 3.4.0 [31], and Statistica version 12 (Stat Soft, Inc., Tulsa, Oklahoma, United States). Pair-wise LD between tested SNPs was computed as both D’ and r² using the genotype data from the tested sample and the Haplovie 4.2 software (http://www.broad.mit.edu/mpg/haplovie/).

Distribution of haplotypes was analyzed by the mentioned above Haplovie 4.2 software. Haplotypes were statistically analyzed if their incidence in the examined group was over 1%. Statistical significance was evaluated using the 1000-fold permutation test.

The analysis of epistatic interactions between tested SNPs was performed by the logistic regression and epistasis option in the PLINK software http://zxx.bwh.harvard.edu/plink/. The false discovery rate (FDR) method was used to correct for multiple comparisons [32, 33].

Results
Patient characteristics
Figure 1 presents the lipid status in the enrolled HD patients.

Lipid-lowering medications (statins, fibrates, or both) were used in 492 patients, of whom 138 (28.0% of total treated) abolished dyslipidemia by both criteria. HD
patients diagnosed as dyslipidemic by K/DOQI criteria [1] (n = 621) did not differ in frequency of lipid-lowering medication compared with subjects without dyslipidemia by the same criteria (n = 587). HD patients showing the atherogenic index ≥3.8 and receiving lipid-lowering medication revealed the TG/HDL-cholesterol ratio of 6.1 (3.8–49.7) (n = 272), whereas HD subjects showing the atherogenic index ≥3.8 and not treated had the TG/HDL-cholesterol ratio of 5.8 (3.8–34.5) (n = 304; P-value 0.047).

Additional file 1: Table S2 shows data of HD patients stratified by serum lipid status. Characteristics of patients dyslipidemic by K/DOQI or the atherogenic index were compared with those of non-dyslipidemic subjects by the respective criterion and with those of non-dyslipidemic patients by both criteria not receiving lipid-lowering medication (Additional file 1: Table S3). Compared to non-dyslipidemic subjects, HD patients dyslipidemic by K/DOQI criteria were the most frequent women, showed higher body mass index (BMI), and lower serum total alkaline phosphatase (ALP) activity. Subjects with atherogenic dyslipidemia revealed more frequently CAD and higher BMI (Additional file 1: Table S2 and Additional file 1: Table S3).

**CASR SNPs and dyslipidemia**

Additional file 1: Table S4 and Additional file 1: Table S5 show statistical analyses of associations between tested CASR SNPs and dyslipidemia. Relationships at P-value < 0.05 for comparisons of the examined dyslipidemic group with a group without dyslipidemia by a respective criterion and also with a non-dyslipidemic group without antilipemic medication were taken for further analyses. Such associations were shown only in the group not receiving lipid-lowering medications. By the Better Associations for Disease and GEnes (BADGE) system [30], there was the fifth-class association between CASR rs7652589 and dyslipidemia diagnosed by the atherogenic index in the dominant model of inheritance. Carriers of the variant allele showed about 1.5-fold higher risk of dyslipidemia diagnosed by the atherogenic index compared with homozygotes of the major allele (Table 1). Prevalence of CAD was not associated with CASR rs7652589 in this group (Additional file 1: Table S6).

HD patients harboring the variant allele of CASR rs1801725 revealed about the 1.7-fold lower risk of dyslipidemia diagnosed by non-HDL-cholesterol ≥130 mg/dL and TG ≥200 mg/dL compared with homozygotes of the major allele (Table 2). Subjects showing homozygosity of the variant allele of CASR rs1801725 revealed 6.8-fold lower prevalence of CAD than those with homozygosity of the major allele as well as the 6.5-fold lower frequency of CAD than those being the major homozygotes and heterozygotes of CASR rs1801725 (Additional file 1: Table S7). All these associations represented the fifth-class by the BADGE system [30].

**Haplotype frequencies and epistatic interactions**

CASR haplotypes and epistatic interactions between CASR, ENHO, RXRA, and LXRA were analyzed in patients not receiving lipid-lowering medication. Dyslipidemic subjects were tested against patients without dyslipidemia by
| Genotypes, MAF, HWE | Patients with atherogenic dyslipidemia | Patients without atherogenic dyslipidemia | Comparison of patients with atherogenic dyslipidemia and without atherogenic dyslipidemia Odds ratio (95% CI), \( P \)-value\(^1\) | Patients without any dyslipidemia | Comparison of patients with atherogenic dyslipidemia and without any dyslipidemia Odds ratio (95% CI), \( P \)-value\(^2\) |
|---------------------|--------------------------------------|------------------------------------------|-------------------------------------------------|--------------------------|-------------------------------------------------|
| GG                  | 91 (32.6)                            | 159 (41.1)                               | Reference                                        | 93 (42.5)                | Reference                                        |
| AG                  | 147 (52.7)                           | 173 (44.7)                               | 1.485 (1.058–2.083), 0.022                       | 91 (41.6)                | 1.651 (1.118–2.438), 0.011                       |
| AA                  | 41 (14.7)                            | 55 (14.2)                                | 1.302 (0.806–2.104), 0.279                       | 35 (16.0)                | 1.197 (0.701–2.046), 0.510                       |
| AA + AG vs GG       | 188 (67.4)                           | 194 (58.9)                               | 1.441 (1.044–1.988), 0.026                       | 126 (57.5)               | 1.525 (1.057–2.200), 0.024                       |
| AA vs GG + AG       | 41 (14.7)                            | 55 (14.2)                                | 1.040 (0.672–1.610), 0.861                       | 35 (16.0)                | 0.906 (0.555–1.479), 0.692                       |
| MAF                 | (0.041)                              | (0.37)                                   | 1.208 (0.966–1.510), 0.0098                      | (0.37)                   | 1.198 (0.926–1.549), 0.169                       |
| \( P \)-value for HWE | 0.138                                | 0.475                                    |                                                   | 0.116                    |                                                   |

Significant differences are indicated using a bold font.

1 – Pearson’s chi-squared test; 2 – Cochran-Armitage trend test

\( P \)-value\(^{\text{a}}\) = 0.095, \( P \)-value\(^{\text{b}}\) = 0.072
### Table 2: CASR rs1801725 and dyslipidemia in patients not receiving lipid-lowering medication

| Genotypes, MAF, HWE | Patients with dyslipidemia diagnosed with non-HDL-cholesterol ≥ 130 mg/dL and TG ≥ 200 mg/dL | Patients without dyslipidemia diagnosed with non-HDL-cholesterol ≥ 130 mg/dL and TG ≥ 200 mg/dL | Comparison of patients with dyslipidemia diagnosed with non-HDL-cholesterol ≥ 130 mg/dL and TG ≥ 200 mg/dL and without dyslipidemia of this type | Patients without any dyslipidemia | Comparison of patients with dyslipidemia diagnosed with non-HDL-cholesterol ≥ 130 mg/dL and TG ≥ 200 mg/dL and without any dyslipidemia |
|---------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------|---------------------------------------------------------------------------------|
|                     | Odds ratio (95% CI), \( P \)-value\(^1\)                                                                 | Odds ratio (95% CI), \( P \)-value\(^1\)                                                                 | Odds ratio (95% CI), \( P \)-value\(^1\)                                                                 | Odds ratio (95% CI), \( P \)-value\(^1\) | Odds ratio (95% CI), \( P \)-value\(^1\)                                                                 |
| GG                  | 92 (78.6)                                                                        | 386 (67.8)                                                                        | Reference                                                                       | 159 (68.5)                  | Reference                                                                       |
| GT                  | 25 (21.4)                                                                         | 169 (29.7)                                                                        | 0.621 (0.385–1.001), \textbf{0.049}                                             | 67 (28.9)                   | 0.645 (0.381–1.091), 0.101                                                     |
| TT                  | 0 (0)                                                                             | 14 (2.5)                                                                           | 0.144 (0.009–2.437), 0.083\(^3\)                                               | 6 (2.6)                     | 0.133 (0.007–2.381), 0.091\(^3\)                                               |
| TT + GT vs GG       | 25 (21.4)                                                                         | 183 (32.2)                                                                         | 0.573 (0.356–0.922), \textbf{0.021}                                             | 73 (31.5)                   | 0.592 (0.351–0.997), \textbf{0.048}                                             |
| TT vs GG + GT       | 0 (0)                                                                             | 14 (2.5)                                                                           | 0.163 (0.010–2.752), 0.144\(^2\)                                               | 6 (2.6)                     | 0.148 (0.008–2.655), 0.185\(^3\)                                               |
| MAF                 | (0.11)                                                                            | (0.17)                                                                             | 0.571 (0.367–0.889), \textbf{0.012}                                             | (0.17)                      | 0.583 (0.361–0.942), \textbf{0.026}                                             |
| \( P \)-value for HWE | 0.195                                                                            | 0.372                                                                             |                                                                                 | 0.736                       |                                                                                 |

Significant differences are indicated using a bold font.

1 – Pearson’s chi-squared test; 2 – Cochran-Armitage trend test, 3 – Fisher’s test

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\( P_{\text{trend}}^2 = 0.010 \); \( P_{\text{genotype}}^1, 3 = 0.026 \)

\( P_{\text{trend}}^2 = 0.023 \); \( P_{\text{genotype}}^1, 3 = 0.060 \)

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the respective criterion or against non-dyslipidemic subjects not receiving lipid-lowering medicines.

Both rs7652589 and rs1801725 SNPs did not show LD: $D' = 0.091$, $r^2 = 0.003$ for the entire HD group (Additional file 1: Figure S1). CASR haplotypes were not associated with dyslipidemia (Additional file 1: Table S8).

Epistatic interactions between tested SNPs, significant in unadjusted analyses, are shown in Table 3. Like other authors [34], we have indicated interactions with FDR $\leq 0.25$. CASR SNPs did not show gene-gene interactions with other tested genes concerning types of dyslipidemia if FDR criterion $\leq 0.25$ was not introduced. Epistatic interaction between $ENHO$ rs2281997 and $RXRA$ rs749759 resulted in about 1.9-fold higher frequency of dyslipidemia diagnosed by LDL-cholesterol $\geq 100$ mg/dL, whereas the interaction between $RXRA$ rs10881578 and $RXRA$ rs749759 correlated with the approximately 2.4-fold lower occurrence of dyslipidemia diagnosed by non-HDL-cholesterol $\geq 130$ mg/dL and TG $\geq 200$ mg/dL.

**CASR SNPs and serum lipids**

In HD patients not receiving lipid-lowering medication ($n = 716$), there were 172 (24.0% of total) subjects showing dyslipidemia by K/DOQI, 113 (15.8% of total) with dyslipidemia by atherogenic index, and 191 (26.7% of total) showing dyslipidemia by both criteria. Non-dyslipidemic patients ($n = 240$) comprised of 33.5% of total. LDL-cholesterol correlated with non-HDL-cholesterol dependently on serum TG levels: Spearman’s rank-order correlation coefficient was 0.962 at TG $< 150$ mg/dL, 0.953 at $\geq 150–250$ mg/dL, and 0.816 at $> 250$ mg/dL (Additional file 1: Figure S2).

Table 4 shows the associations of CASR SNPs with serum lipids in HD patients not receiving lipid-lowering medication. The atherogenic index (the TG/HDL-cholesterol ratio) as a continuous variable correlated with CASR rs7652589 in the dominant and additive models of inheritance. Increased serum TG levels predominantly participated in the higher atherogenic index in homozygotes for the variant rs7652589 allele. Such an analysis for rs1801725 did not reveal significant associations with serum lipids (Additional file 1: Table S9).

**CASR SNPs as correlates of dyslipidemia and CAD among other variables**

We have analyzed whether CASR SNPs shown as associated with serum lipid status and CAD remained relevant also among parameters significantly differing HD subjects

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|----------------------------|
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| Table 3 | Interactions between tested SNPs significant in unadjusted analyzes in HD patients not receiving lipid-lowering medication |
|---------|--------------------------------------------------------------------------------------------------------------------------|
| CHR1    | GENE1 | SNP1 | CHR2 | GENE2 | SNP2 | The odds ratio for interaction | Chi-square | P-value | FDR-adjusted P-value |
|---------|-------|------|------|-------|------|-------------------------------|------------|---------|---------------------|
| Patients with dyslipidemia by K/DOQI vs. patients without this phenotype |
| 9       | ENHO  | rs2281997 | 9    | RXRA  | RXRA | 1.7690 | 7.007 | 0.0081 | 0.3645 |
| Patients with LDL-cholesterol $\geq 100$ mg/dL vs. patients without this phenotype |
| 3       | CASR  | rs1801725 | 9    | RXRA  | rs10881578 | 0.6030 | 4.048 | 0.0442 | 0.9916 |
| 9       | ENHO  | rs2281997 | 9    | RXRA  | rs749759 | 1.8920 | 8.794 | 0.0030 | 0.1350 |
| Patients with non-HDL-cholesterol $\geq 130$ mg/dL and TG $\geq 200$ mg/dL vs patients without this phenotype |
| 9       | ENHO  | rs2281997 | 11   | LYRA  | rs2279238 | 2.4220 | 5.962 | 0.0146 | 0.3285 |
| 9       | RXRA  | rs10881578 | 9    | RXRA  | rs749759 | 0.4213 | 9.829 | 0.0017 | 0.0765 |
| Patients with atherogenic index $\geq 3.8$ vs. patients without this phenotype |
| 3       | CASR  | rs7652589 | 9    | ENHO  | rs72735260 | 0.5744 | 4.680 | 0.0305 | 0.8595 |
| 9       | ENHO  | rs2281997 | 11   | LYRA  | rs2279238 | 1.7750 | 4.298 | 0.0382 | 0.8595 |
| Patients with dyslipidemia by K/DOQI vs. patients without dyslipidemia by all used criteria |
| 9       | ENHO  | rs2281997 | 9    | RXRA  | rs749759 | 1.767 | 5.528 | 0.0187 | 0.8415 |
| Patients with LDL-cholesterol $\geq 100$ mg/dL vs. patients without dyslipidemia by all used criteria |
| 9       | ENHO  | rs2281997 | 9    | RXRA  | rs749759 | 1.945 | 6.839 | 0.0089 | 0.4005 |
| Patients with non-HDL-cholesterol $\geq 130$ mg/dL and TG $\geq 200$ mg/dL vs. patients without dyslipidemia by all used criteria |
| 9       | ENHO  | rs2281997 | 11   | LYRA  | rs2279238 | 2.604 | 5.017 | 0.0251 | 0.5648 |
| 9       | RXRA  | rs10881578 | 9    | RXRA  | rs749759 | 0.485 | 5.743 | 0.0166 | 0.5648 |
| Patients with atherogenic index $\geq 3.8$ vs. patients without dyslipidemia by all used criteria |
| 3       | CASR  | rs7652589 | 9    | ENHO  | rs72735260 | 0.564 | 3.904 | 0.0482 | 0.8162 |

P-values adjusted for FDR equal to or below 0.25 are considered significant and are indicated using a bold font.

Abbreviations: CHR1 Chromosome of first SNP, SNP1 Identifier for first SNP, GENE1 Gene of the first SNP, CHR2 Chromosome of second SNP, SNP2 Identifier for second SNP, GENE2 Gene of the second SNP, P-value 1df asymptotic P-value, FDR-adjusted P-value P-value adjusted for false discovery rate.
as shown in Additional file 1: Table S3 [gender, age, diabetic nephropathy, renal replacement therapy (RRT) duration, CAD, BMI, and ALP activity]. For CAD, we have also included dyslipidemia diagnosed by non-HDL-cholesterol ≥130 mg/dL and TG ≥200 mg/dL [1] as a possible explanatory variable. We performed all analyses in patients not receiving lipid-lowering medication.

Variables significantly associated with dyslipidemia diagnosed by atherogenic index [2] included BMI (OR 1.13, 95%CI 1.07–1.19, P = 0.20E-6), RRT duration (OR 1.06, 95%CI 1.02–1.10, P = 0.007), and CASR rs7652589 in dominant model of inheritance (OR 1.50, 95%CI 1.03–2.24, P = 0.048). The atherogenic index expressed as a continuous variable correlated independently with CASR rs7652589 in dominant model of inheritance (β = 0.10 ± 0.05, P = 0.021) together with BMI (β = 0.26 ± 0.05, P = 1.1E-8) and diabetic nephropathy (β = 0.09 ± 0.05, P = 0.044). However, an association of rs7652589 with serum TG concentrations was borderline (β = 0.08 ± 0.05, P = 0.083) among other tested variables of which only BMI correlated significantly with TG (β = 0.25 ± 0.05, P = 5.2E-8).

Dyslipidemia diagnosed by non-HDL-cholesterol ≥130 mg/dL and TG ≥200 mg/dL [2] showed an association with BMI (OR 1.12, 95%CI 1.06–1.19, P = 1.7E-4), male gender (OR 0.41, 95%CI 0.24–0.72, P = 0.002), and CASR rs1801725 in dominant model of inheritance (OR 0.52, 95%CI 0.28–0.98, P = 0.042). CASR rs1801725 did not reveal an independent correlation with CAD (OR 0.26, 95%CI 0.03–2.34, P = 0.231), Age (OR 1.04, 95%CI 1.02–1.06, P = 1.2E-4), diabetic nephropathy (OR 2.02, 95%CI 1.11–3.69, P = 0.021), and BMI (OR 1.07, 95%CI 1.01–1.14, P = 0.024) were significant explanatory variables for CAD among HD patients not receiving lipid-lowering medication.

**CASR SNPs and lipid-lowering treatment**

There were no differences in the distribution of CASR SNPs in HD subjects who showed specific types of dyslipidemia despite lipid-lowering medication and those who did not demonstrate this kind of dyslipidemia or were non-dyslipidemic without lipid-lowering therapy (Additional file 1: Table S4 and Additional file 1: Table S5).

**Correlations of CASR, ENHO, RXRA, and LXRA transcripts**

In unadjusted analyses, transcripts of tested genes correlated significantly with RRT duration (CASR, ENHO), dry body weight (RXRA, LXRA), BMI (RXRA, LXRA), and serum albumin concentration (LXRA, ENHO) (Additional file 1: Table S10). If all tested variables were adjusted concerning RRT duration, dry body weight, and albumin level, as appropriate, CASR transcript correlated positively and

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Table 4 CASR rs7652589 polymorphic variants and serum lipids in HD patients not receiving lipid-lowering medication (n = 666)

| Parameter             | GG     | AG     | AA     | Model of inheritance | P-value<sup>1</sup> | P-value<sup>2</sup> |
|-----------------------|--------|--------|--------|-----------------------|---------------------|---------------------|
|                       | n = 250| n = 320| n = 96 |                       |                     |                     |
| Total cholesterol, mg/dL | 171.5 (72–282) | 174.5 (65–363) | 164.5 (92–296) | AG + AA vs. GG | 0.883 | 0.024 |
|                       |        |        |        | AA vs. GG + AG | 0.311 | 0.420 |
|                       |        |        |        | AA vs. GG | 0.442 | 0.097 |
| HDL-cholesterol, mg/dL | 42 (6–94) | 39 (10–118) | 39 (17.3–82) | AG + AA vs GG | 0.011 | 0.086 |
|                       |        |        |        | AA vs. GG + AG | 0.376 | 0.455 |
|                       |        |        |        | AA vs. GG | 0.070 | 0.412 |
| Triglycerides, mg/dL  | 131.3 (40–585) | 140.5 (35–1105) | 140 (35–406) | AG + AA vs GG | 0.067 | 0.027 |
|                       |        |        |        | AA vs. GG + AG | 0.680 | 0.284 |
|                       |        |        |        | AA vs. GG | 0.671 | 0.061 |
| LDL-cholesterol, mg/dL| 94.6 (27.8–208.4) | 98 (20–350) | 92.5 (27–369) | AG + AA vs. GG | 0.705 | 0.126 |
|                       |        |        |        | AA vs. GG + AG | 0.480 | 0.545 |
|                       |        |        |        | AA vs. GG | 0.612 | 0.239 |
| Non-HDL-cholesterol, mg/dL | 125 (52–234) | 132 (8–282) | 120 (58–262) | AG + AA vs. GG | 0.430 | 0.037 |
|                       |        |        |        | AA vs. GG + AG | 0.432 | 0.332 |
|                       |        |        |        | AA vs. GG | 0.737 | 0.079 |
| TG/HDL-cholesterol ratio | 3.1 (0.6–30.8) | 3.6 (0.4–34.5) | 3.4 (0.5–15) | AG + AA vs. GG | 0.015 | 0.037 |
|                       |        |        |        | AA vs. GG + AG | 0.971 | 0.177 |
|                       |        |        |        | AA vs. GG | 0.325 | 0.041 |

Conversion factors to SI units are as follows: for cholesterols – 1 mg/dL = 0.0259 mmol/L, for triglycerides – 1 mg/dL = 0.0113 mmol/L.

Significant differences are indicated using a bold font.

1 – Mann Whitney test; 2 – P-value for rs7652589 SNP in a linear regression model including gender, age, BMI, diabetic nephropathy, coronary artery disease, and alkaline phosphatase activity.
ENHO transcript inversely with RRT duration. Patients on RRT > 5 years (n = 53) showed relative CASR transcript level of 0.470 (0.056–7.976), whereas subjects on RRT < 1 year (n = 20) had CASR transcript of 0.181 (0.041–3.702) (P = 0.010, Mann-Whitney test). Higher body mass correlated positively with RXRA and CASR transcript levels, and negatively – with LXRA transcript amounts. Serum albumin positively correlated with ENHO transcript. LXRA transcript was positively associated with CAD at the borderline level of significance. Tested transcripts did not correlate with gender, age, diabetic nephropathy, types of dyslipidemia, active HBV/HCV infection, liver enzyme activities but ALP with RXRA transcript, inflammatory state assessed by plasma C-reactive protein, or lipid-modifying treatment (Additional file 1: Table S11).

In univariate analysis, CASR transcript was lower at a borderline significance level in patients harboring the rs1801725 variant allele compared with homozygotes of the major allele (0.20, 0.06–7.80 vs. 0.43, 0.04–5.06, P = 0.058). For RXRA rs10776909, associations between polymorphic variants and transcript levels were borderline in the additive and recessive inheritance models in univariate analysis and also after adjustment for RRT duration, dry body weight, and albumin level. Homozygosity of the variant allele in LXRA rs7120118 was associated with lower transcript levels, significantly in the recessive model of inheritance in univariate analysis and also after adjustment for RRT duration, dry body weight, and albumin level (Additional file 1: Table S12).

Relative levels of transcripts correlated with one another. CASR transcript positively correlated with RXRA, LXRA, and ENHO transcripts in patients not receiving lipid-lowering medication (Table 5).

After adjustment for RRT duration, dry body weight, and serum albumin level, there were no significant correlations between relative CASR transcript amounts and serum lipids or the TG/HDL-cholesterol ratio. A positive correlation was demonstrated between ENHO transcript and LDL-cholesterol in the entire studied group (P-value 0.023), and a negative correlation with TG among HD patients not receiving lipid-lowering medication (P-value 0.028) (Additional file 1: Table S13). In patients taking lipid-modifying medicines, there were positive correlations between ENHO transcript and total cholesterol (P-value 0.044) and HDL-cholesterol (P-value 0.032).

### Discussion

In the studied HD patients, dyslipidemia occurred in 80.1% of subjects, what is in full agreement with previous reports [1]. In HD subjects like in healthy men [35], a correlation between LDL-cholesterol and non-HDL-cholesterol depended on TG levels, worsening with higher serum TG concentrations.

There were associations between CASR SNPs (rs7652589, rs1801725) and dyslipidemia in HD patients not receiving lipid-modifying medications. During treatment with lipid-lowering medications, these correlations are not observed, maybe because not unified protocol of lipid-modifying therapy was used concerning the initiation of such treatment as

| Gene | Relative transcript amount Median (min-max) | RXRA | LXRA | ENHO |
|------|------------------------------------------|------|------|------|
| All tested HD patients (n = 112) | | | | |
| CASR | 0.320 (0.041–7.976) | 0.001; 0.001 | | |
| RXRA | 0.850 (0.095–2.813) | | | |
| LXRA | 1.014 (0.012–7.117) | | | |
| ENHO | 0.666 (0.030–2.524) | | | |
| Patients not receiving lipid-lowering treatment (n = 77) | | | | |
| CASR | 0.315 (0.041–7.976) | 0.002; 0.003 | | |
| RXRA | 0.875 (0.095–2.813) | | | |
| LXRA | 0.968 (0.012–7.117) | | | |
| ENHO | 0.752 (0.030–2.460) | | | |
| Patients receiving lipid-lowering treatment (n = 35) | | | | |
| CASR | 0.395 (0.065–4.050) | 0.857; 0.987 | | |
| RXRA | 0.751 (0.162–1.590) | | | |
| LXRA | 1.074 (0.071–1.977) | | | |
| ENHO | 0.531 (0.055–2.524) | | | |

Significant correlations are indicated using a bold font.
well as types and doses of medications. Therefore, effects of treatment could be hardly comparable concerning CASR SNPs.

HD patients bearing the variant allele of CASR rs7652589 showed approximately 1.5-fold higher frequency of the atherogenic index equal to or exceeding 3.8, that is values assigned to atherogenic dyslipidemia [2], more elevated TG/HDL-cholesterol ratio, and higher serum TG concentrations. It is worthy to note that the relationship between rs7652589 and atherogenic dyslipidemia was also significant among clinical and laboratory variables tested together. Mentioned above associations were not accompanied by a higher prevalence of CAD in subject harboring the variant allele of rs7652589 SNP. Although atherogenic serum lipid profile is associated with atherosclerosis [36] and CAD [37], our previous retrospective observational study [7] and the 7-year prospective study [38] did not show an influence of CASR rs7652589 on all-cause, cardiac or cardiovascular mortality of HD patients, what could be expected for patients bearing the variant allele of rs7652589 that is associated with atherogenic dyslipidemia.

SNP rs1801725 was not directly associated with concentrations of individual lipid components, but the simultaneous occurrence of non-HDL-cholesterol ≥130 mg/dL and TG ≥ 200 mg/dL was showed with about 1.7-fold lower frequency among bearers of the variant allele not receiving lipid-lowering medications. The level of non-HDL-cholesterol is used as a surrogate for increased remnant lipoproteins and apolipoprotein B, at least in normolipidemic individuals [35]. If TG levels are high (≥ 200 mg/dL), much of the non-HDL-cholesterol is very-low-density lipoprotein and intermediate-density lipoprotein remnants, but an association with apolipoprotein B is less strong [39]. In this study, LDL-cholesterol yielded lower correlation with non-HDL-cholesterol at higher serum TG levels. However, further studies might have been recommended to answer whether directly determined circulating lipoprotein remnants, and apolipoprotein B correlate with rs1801725 in HD patients. The variant allele of CASR rs1801725, which was associated independently with the more favorable serum lipid profile (the lower coincidence of non-HDL-cholesterol ≥130 mg/dL and TG ≥ 200 mg/dL), showed a borderline negative correlation with CASR transcript level additionally. A significance of this finding concerning dyslipidemia and its atherosclerotic consequences is unknown. CASR transcript and dry bodyweight show a positive correlation. Subjects with obesity defined by BMI > 30 kg/m² expressed higher levels of CASR transcript in the liver, and CaSR was proposed as a contributor to obesity-associated hepatic metabolic consequences [14]. Maybe, lower levels of CASR transcript in subjects harboring the variant allele of rs1801725 contribute to the more favorable metabolic profile in HD patients, but direct associations between CASR transcript, types of dyslipidemia, CAD, and serum lipids were not found in our study.

The regulatory region of the human CASR (chromosome 3q14.3–21) includes two promoters (promoter 1 and 2) that encode for two alternative 5′-untranslated regions. CASR has seven exons [40]. Because CASR rs1801725 SNP is located in exon 7, it probably cannot directly affect the binding of transcription factors involved in dyslipidemia and CAD as it was shown for rs7652589 SNP [7]. The exonic SNP rs1801725 might be acting through the nonsynonymous exchange of alanine to serine at position 986 in the CaSR cytoplasmic tail. This exchange was initially referred to as associated with the production of a less active receptor by the variant allele of rs1801725 [41, 42]. However, two functional studies documented the normal activity of CaSR coded by the variant allele of rs1801725 SNP [43, 44]. Therefore, the impact of the variant rs1801725 allele cannot be simply explained by a less active CaSR. In our study, the nonsynonymous exchange of alanine to serine was shown together with lower relative CASR transcript amount at a borderline level of significance in the dominant model of inheritance in univariate analysis. However, in general, amino acid substitution alters the function (quality) of proteins but not the amount. Thus, lower relative CASR transcript amount could not be explained by an impact of rs1801725. Vezzoli et al. [10] designed two constructs containing A (major) or G (variant) allele at the rs6776158 SNP in the CASR promoter 1 and found that promoter 1 including the G allele showed lower transcriptional activity than that with the A allele. In light of this finding, it seems reasonable to investigate in further studies the role of rs6776158 and haplotypes formed by rs6776158 and rs1801725 variants concerning dyslipidemia and related comorbidities in HD patients.

It has to be stressed, however, that the fifth-class associations by the BADGE system [30], like those between CASR rs7652589 and atherogenic dyslipidemia, CASR rs1801725 and dyslipidemia diagnosed by non-HDL-cholesterol ≥130 mg/dL and TG ≥ 200 mg/dL, and CASR rs1801725 and CAD prevalence, do not provide assurance of reproducibility. Further studies are needed to confirm direct associations between CASR SNPs, serum lipid abnormalities, and CAD.

RXR heterodimerizes with PPARγ [19], which was found to be activated by up-regulation of CaSR [18]. This co-action may explain the positive correlation between CASR and RXRA transcripts. RXRa and LXRα form the heterodimer LXRα-RXRa [20]. Thus RXRA and LXRα transcripts may positively correlate, as we have shown in HD patients. However, much stronger was a
positive correlation between RXRA and ENHO transcripts. In HD subjects, associations were found between ENHO rs2281997 and dyslipidemia [22]. In this study, ENHO SNPs (rs2281997, rs72735260) did not correlate with ENHO transcript levels. It may suggest an association of ENHO transcript with other ENHO SNP(s) than rs2281997 or rs72735260. However, serum LDL-cholesterol positively correlated with ENHO transcript and ENHO rs2281997 and RXRA rs7497759 showed epistatic interaction concerning dyslipidemia diagnosed by LDL-cholesterol ≥100 mg/L. Up-regulation of ENHO under dyslipidemic conditions is expected to increase the production of adropin, a protein product of ENHO, which is a factor governing glucose and lipid homeostasis [21]. In HD patients, homozygotes of the major allele in ENHO rs2281997 were suggested to have higher circulating adropin [27]. In Behçet’s disease, serum adropin level correlated positively with LDL-cholesterol [45]. Therefore, ENHO transcript level and adropin were both found as positively associated with LDL-cholesterol. In patients not receiving lipid-lowering treatment, adjustment for body mass, serum albumin level and RRT duration revealed correlation also between CASR and ENHO transcripts.

Kumar et al. [21] have shown that adropin is associated with suppression of lipogenic gene expression. Inversely, stimulation of LXRA suppresses hepatic ENHO expression [21]. We have determined transcripts of tested genes in PBMCs, not in the liver, and demonstrated a borderline negative association between ENHO transcript levels and LXRA transcripts in unadjusted analyses, what, however, is in agreement with findings of Kumar et al. [21]. ENHO transcript levels negatively correlated with serum TG in the studied HD group and subjects with Behçet’s disease [45] as well as plasma adropin negatively associated with TG and atherogenic index in our previous study on HD patients [22]. However, CASR transcript showed a weaker association with ENHO transcript than that which was found for RXRA and LXRA transcripts.

Advanced secondary hyperparathyroidism, atherosclerotic plaques, and vascular calcifications frequently occur together in uremic patients [3]. As demonstrated in Additional file 1: Table S2 and Additional file 1: Table S3, higher serum parathyroid hormone (PTH) levels were shown in HD patients with atherogenic dyslipidemia compared with PTH concentrations in HD subjects without this type of dyslipidemia, however, only at the borderline level of significance (P = 0.067). In the study by Mitwalli et al. [46], hyperparathyroid dialysis patients had significantly higher serum TG levels compared with subjects without hyperparathyroidism what speaks in favor for the correlation between secondary hyperparathyroidism and atherogenic dyslipidemia.

We previously found that homozygosity in the variant allele (A) of CASR polymorphism rs7652589 is associated with more severe secondary hyperparathyroidism [7]. In this study, carriers of the rs7652589 variant allele showed a higher risk of dyslipidemia diagnosed using the atherogenic index. Therefore, secondary hyperparathyroidism and atherogenic dyslipidemia have a common genetic background as CASR polymorphism rs7652589. Our study implicates that activating CaSR for treatment of secondary hyperparathyroidism, we may suspect that also the severity of atherogenic dyslipidemia will be ameliorated, at least in subjects harboring the rs7652589 risk allele. Serum lipid profile is worth to be monitored during calcimimetic administration, the best in patients previously genotyped for CASR SNPs.

On the other hand, secondary hyperparathyroidism aggravates with prolongation of dialysis treatment [47]. Inversely, serum lipid profile is not worse, if not slightly better, in patients treated with HD > 5 years compared with lipid profile in subjects dialyzed < 1 year [46]. In the group tested for CASR transcript, median serum PTH concentration was 2.2-fold higher in patients being on RRT > 5 years compared with PTH in subjects being on RRT < 1 year. This finding occurs together with 2.6-fold higher relative CASR transcript level in patients being on RRT > 5 years. However, CASR transcript in PBMCs was not associated either with PTH or serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, TG).

Higher relative CASR transcript levels may not correspond with CaSR activation and function, because mRNA CASR maturation and stability, translation, insertion of the CaSR protein into cell membrane, and CaSR turnover may be disturbed as postulated by Garner et al. [48] basing on their study on parathyroid adenomas from patients with primary hyperparathyroidism. In their study, there were no correlations between parathyroid mRNA CASR and serum PTH or ionized calcium concentrations [48]. Clinically, aggravation of secondary hyperparathyroidism in long-term dialysis patients is followed by more frequent use of calcimimetics for the amelioration of hyperparathyroidism consequences [49].

Our study did not include HD patients receiving calcimimetic medication. Cinacalcet decreased TG content in adipocytes (line LS14 derived from a human metastatic liposarcoma) by 20% through CaSR activation but CaSR stimulation in HepG2 cells exhibited a 19% increased TG content in the presence of oleic acid and elevation in the expression of proinflammatory factors [14]. In omental adipose tissue obtained from individuals without end-stage renal disease, the CASR rs1042636 major homozygosity (AA) was associated with a lower frequency of CaSR responsiveness to the antilipolytic effect of cinacalcet, whereas the rs1042636 variant allele (G)
was associated with a greater antilipolytic frequency. Therefore, a suppressive action of cinacalcet on free fatty acid release may be less pronounced in the rs1042636 AA homozygotes. An analysis of the same study samples performed concerning the rs1801725 genotypes yielded no conclusive results [17]. In our study, CASR rs7652589 and rs1801725 correlated with dyslipidemia in HD patients but their simultaneous associations with calcimimetics need to be shown in future studies.

Conclusions
1. In HD patients, CASR polymorphisms (rs7652589, rs1801725) play a noticeable role in dyslipidemia. CASR is associated with RXRA, LXRA, and ENHO at the transcript level.
2. Relative CASR transcript level positively correlates with dry body weight, RXRA, LXRA and ENHO transcripts, and RRT duration, but is not dependent on gender, age, diabetic nephropathy, types of dyslipidemia, or lipid-modifying treatment.
3. Further investigations may elucidate whether other CASR SNPs contribute to associations shown in this study.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12882-019-1619-0.

Additional file 1. Supplementary material (tables and figures) contains characteristics of the analyzed polymorphisms, data of patients, associations between CASR polymorphisms and haplotypes with dyslipidemia, correlates of CASR, RXRA, LXRA, and ENHO transcript amounts, the LD data, and correlations between serum cholesterol and serum TG levels.

Abbreviations
ALP: Alkaline phosphatase; BADGE: Better Associations for Disease and GEnes; BMI: Body mass index; CAD: Coronary artery disease; CASR: Calcium-sensing receptor gene; CaSR: Calcium-sensing receptor; ENHO: Endocrine homeostasis-associated gene; FDR: False discovery rate; HD: Hemodialysis; HDL: High-density lipoprotein; HLM: High-resolution melting curve; HWE: Hardy-Weinberg equilibrium; K/DOQI: National Kidney Foundation/Kidney Disease Outcomes Quality Initiative; LD: Linkage disequilibrium; LDL: Low-density lipoprotein; LXRA: Liver X receptor; RXRA: Liver X receptor α gene; MAF: Variant allele frequency; Mi: Myocardial infarction; PBMC: Peripheral blood mononuclear cell; PPARY: Peroxisome proliferator-activated receptor γ; RRT: Renal replacement therapy; RXR: Retinoid X receptor; RXRA: Retinoid X receptor α gene; SNP: Single nucleotide polymorphism; TC: Total cholesterol; TG: Triglycerides

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Authors’ contribution
AEG conceived the study, designed the research, was involved in the data collection, analyzed the data, participated in funding for the project. BAF performed analyses of CASR, ENHO, RXRA, and LXRA relative transcript levels. PPJ was involved in the data collection and performed statistical analysis. LN was involved in the data collection. AM was responsible for the genotyping. PPJ was responsible for the genotyping, participated in funding for the project. All authors edited and approved the final version of the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The Institutional Review Board of the Poznan University of Medical Sciences, Poland, approved the research design (Act no 892/17). The research was conducted ethically following the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants included in the study or their parents, as appropriate.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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