Prediction of steroid resistance and steroid dependence in nephrotic syndrome children

Katarzyna Zaorska1*, Piotr Zawierucha2, Monika Świerczewska1, Danuta Ostalska-Nowicka3, Jacek Zachwieja3 and Michał Nowicki1

Abstract
Background: Steroid resistant (SR) nephrotic syndrome (NS) affects up to 30% of children and is responsible for fast progression to end stage renal disease. Currently there is no early prognostic marker of SR and studied candidate variants and parameters differ highly between distinct ethnic cohorts.

Methods: Here, we analyzed 11 polymorphic variants, 6 mutations, SOCS3 promoter methylation and biochemical parameters as prognostic markers in a group of 124 Polish NS children (53 steroid resistant, 71 steroid sensitive including 31 steroid dependent) and 55 controls. We used single marker and multiple logistic regression analysis, accompanied by prediction modeling using neural network approach.

Results: We achieved 92% (AUC = 0.778) SR prediction for binomial and 63% for multinomial calculations, with the strongest predictors ABCB1 rs1922240, rs1045642 and rs2235048, CD73 rs9444348 and rs4431401, serum creatinine and unmethylated SOCS3 promoter region. Next, we achieved 80% (AUC = 0.720) in binomial and 63% in multinomial prediction of SD, with the strongest predictors ABCB1 rs1045642 and rs2235048. Haplotype analysis revealed CD73_AG to be associated with SR while ABCB1_AGT was associated with SR, SD and membranoproliferative pattern of kidney injury regardless the steroid response.

Conclusions: We achieved prediction of steroid resistance and, as a novelty, steroid dependence, based on early markers in NS children. Such predictions, prior to drug administration, could facilitate decision on a proper treatment and avoid diverse effects of high steroid doses.

Keywords: Nephrotic syndrome, Prediction modeling, Single nucleotide polymorphisms, Steroid dependence, Steroid resistance

Background
Childhood nephrotic syndrome (NS) is characterized by massive proteinuria exceeding 40 mg/m²/hr, generalized edema and hypoalbuminemia. Its prevalence is 12–16/100 000 and the underlying cause is idiopathic in 95% of cases [1]. Most patients that respond well to the standard first-line treatment with corticosteroids are defined as steroid sensitive (SS), while 20%–30% that fail to respond are defined as steroid resistant (SR), therefore, are more difficult to treat and 36%–50% of them progress to end-stage renal disease within 10 years. Also, 60%–70% of initially sensitive patients will develop steroid dependence (SD), frequent relapses or secondary steroid resistance [2, 3]. So far, steroid resistant subtype of NS in children has been correlated with male sex, young age of onset, focal segmental glomerulosclerosis (FSGS) on kidney biopsy and genetic variants including single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) identified in over 53 genes [4–7]. However, many of those parameters are highly divergent between patients of different ethnicities. Differences in genetic inheritance
models and plethora of definitions used by the researchers, as well as heterogeneity of nephrotic syndrome and its subphenotypes themselves, make the comparisons among the studies interesting, yet challenging and constricted. Despite extensive research, there is no early predictor of steroid unresponsiveness that could be clinically useful. The patient’s actual response to steroid treatment and renal histopathology are, so far, the foremost guidelines for clinicians to rely on and for a long-term prognosis. Still, both are invasive and expose patients to wide spectrum of side effects [1, 3, 8].

Here, we present the results of prediction modeling using neural network approach and multifactorial analysis including genetic and epigenetic variables in a cohort of Polish children with nephrotic syndrome. It is an attempt to predispose the type of steroid response and assess a prognosis for patients on the basis of factors characteristic to that ethnic population.

Methods
Patients and study design
In total, 124 patients with NS and 55 healthy controls were analyzed in this study. Of these, 75 NS patients (40 SR and 35 SS) and 32 controls comprised the N1-set and were samples used in our previous study [9]. From the Clinic of Cardiology and Nephrology, University of Medical Sciences in Poznan, Poland, we recruited 49 patients newly diagnosed with NS (13 SR and 36 SS) and 23 controls in 2017–2018, and they comprised the N2-set. All participants were of Polish ethnic origin, from Wielkopolska region, and from the same hospital centre, therefore both sets were pooled together for statistical analysis inference and prediction modeling purposes. All patients were submitted to glucocorticosteroid treatment as a first line therapy. They were further assigned to subgroups upon their initial response to steroid treatment, according to the ISKDC definitions and guidelines [10]. Briefly, steroid sensitivity was defined as a complete remission within initial 4 weeks of treatment, steroid dependence—2 consecutive relapses during therapy, or within 2 weeks of ceasing therapy, primarily steroid sensitivity (PSS)—no relapses during initial 4 weeks of treatment, and steroid resistance—failure to achieve complete remission after 8 weeks of corticosteroid therapy. Thus, the samples were divided into the following subgroups: healthy controls and NS patients, comprising SR and SS, further divided into SD and PSS. Since we analyzed SOCS3 CpG region of the N1-set in the previous study, here only the N2-set was subjected to methylation-specific PCR. Both sets were genotyped for 16 SNPs and 1 CNV. The study design is presented, in brief, in Fig. 1.

Laboratory parameters measured at disease onset were collected from the patient’s documentation (when available) and used for statistical analysis. eGFR was determined according to the Schwartz formula. The ranges for all studied parameters were evaluated considering the reference values for age and sex of the patients.

Sample collection
Peripheral blood was collected in EDTA tubes from all the patients at the first episode of NS before drug administration. Genomic DNA was purified using ExtractME DNA Blood Kit (Blirt S.A.) according to the manufacturer’s instructions and stored at −20 °C.

Methylation analysis
72 samples (the N2-set) were subjected to the methylation-specific PCR for two SOCS3 CpG regions (−1070/−926 bp and −526/−285 bp, relative to ATG triplet) as described in the previous study [9].

Genotyping
In total, 179 samples were genotyped for 1 CNV polymorphism i.e. rs5844572 and 16 single nucleotide changes, including 10 autosomal SNPs and 6 point mutations (5 autosomal, 1 mitochondrial), in 8 genes, i.e.: rs1922240, rs1045642 and rs2235048 in ABCB1, rs2070767, rs2000466 in MIF, rs37972 in GLCCI1, rs3124591 and rs139994842 in NOTCH1, rs9444348 and rs4431401 in CD73, rs730882194, rs587777482 and rs587777481 in EMP2, rs74315342 and rs1057516414 in NPHS2, and rs199474657 in MT-TL1. All 16 single nucleotide changes were amplified and genotyped in two separate multiplex reactions, encompassing as follows: rs1922240, rs1045642, rs2235048, rs2070767, rs2000466, rs139994842, rs9444348, rs730882194 in one reaction, and rs37972, rs3124591, rs4431401, rs587777482, rs587777481, rs74315342, rs1057516414 and rs199474657 in the other reaction. Each reaction was performed in a 10-μl mixture containing 1 ng genomic DNA, 1U FastStart Taq Polymerase, 1xPCR buffer with
1.5 mM MgCl₂, 1 × GC-rich buffer, 200 μl of dNTPs (all components from Roche) and a proper concentration of primers’ mixture. Specifics for 16 variants with primers’ sequences and concentrations are shown in Additional file 1: Table S1. Thermocycling was conducted under conditions: 95 °C for 10 min for 1 cycle, 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s for 30 cycles, followed by 72 °C for 15 min. The PCR products were cleaned using ExoI/rSAP mixture (New England Biolabs) according to the manufacturer’s instructions. Next, single base extension (sbe) PCR was performed, using 1.5 μl of SNaPshot reaction mix (Applied Biosystems), a proper concentration of sbe primers and 1 μl of cleaned product, in a total volume of 5 μl under conditions: 96 °C for 2 min, 96 °C for 10 s, 50 °C for 5 s, 60 °C for 30 s for 25 cycles. The sbe-PCR products were cleaned using rSAP (New England Biolabs) and run on ABI3130 Genetic Analyzer under conditions: the injection voltage of 2.5 kV for 10 s, run time of 600 s at 60 °C, with POP-7 and on a 36-cm capillary length array.

CNV (rs5844572) was genotyped in the PCR with Forward primer labeled with the fluorescent tag 6-FAM. The sequences of the primers were: Forward-6-FAM 5′-CTT GTCCTCTTTCCGTATGTC-3′ and Reverse 5′-ACT CGGGGGACATCACGC-3′. The 10 μl reaction mixture contained 200 nM of each primer, 200 μM of each dNTP, 1U FastStart Taq Polymerase (Roche), 1xPCR buffer with 1.5 mM MgCl₂, and 5 ng genomic DNA. The conditions were: 95 °C for 4 min, 95 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min for 35 cycles, followed by 72 °C for 7 min. The PCR product was cleaned using ExoI/rSAP mixture (New England Biolabs) according to the manufacturer’s instructions and run on ABI3130 Genetic Analyzer with parameters: POP-7 polymer, 36-cm capillary array, injection time 16 s, injection voltage 1.2 kV, run time 1200 s at 60 °C. The position of the result peaks were as follows: 116.5 bp for CATT₅, 120.5 bp for CATT₆, and 124.5 bp for CATT₇.

GeneMapper v4.0 (Applied Biosystems) was used for allelic discrimination. All primers were designed using web-based software BatchPrimer3 v1.0, and sbe primers were verified using OligoAnalyzer v3.1. Genes’ sequences and SNPs’ and mutations’ information were acquired from Ensembl Genome Browser. SNPs’ and mutations’ information and ‘rs’ numbers were verified with Variant Validator.

Statistics
Categorical variables were presented as frequencies with percentages and were analyzed using chi-square and Fisher’s exact tests, whereas continuous variables were presented as mean values with standard deviation and were analyzed using multiple comparison tests. We used the Kolmogorov–Smirnov test to determine the distribution normality of continuous variables. One-way two-sided ANOVA with Holm correction [11], and Kruskal–Wallis test with Dunn correction based on the superior false discovery rate procedure [12], were applied in case of normal and non-normal data distribution, respectively.

The Fisher’s exact test was used for allele and genotype frequencies comparison under the allelic, dominant, recessive and over-dominant genetic models and for methylation patterns of two SOCS3 promoter fragments (SOCS3.1 and SOCS3.2) comparison between study subgroups. Deviation from HWE was estimated using chi-square test. To provide more powerful genotype-based test for association we performed logistic regression analysis using the Cochran-Armitage trend test under the allelic, dominant, recessive and additive models, with the additional testing of additivity of multiplicative model with the reference minor allele. Specifics and correlation of each genetic model are described in detail elsewhere [13]. All logistic regression parameters were calculated in PLINK. P-value ≤ 0.05 was considered statistically significant. The haplotype analysis and pairwise linkage disequilibrium (LD) were performed using SHEsis software [14].

Advanced prediction modeling was assessed with Neural Network (NN) approach. In brief, Neural Network is an algorithm that works in a manner resembling the one of neurons in a human brain. It consists of three layers, an input, hidden and output layer. The input layer represents the data used as prediction variables and the output layer represents the model’s prediction (the summary effect of all incoming factors). The key component is the hidden layer, where the input data is modified and given weights, forming a set of nodes called neurons. On the basis of several internal functions the system is trained to self-learn the relationship between the labels and the variables, during multiple discrete steps (iterations), each time calculating and updating an error to produce the best final prediction. In total, 17 variables (9 SNPs, 2 mutations, 1 CNV, 2 methylation status and 3 demographics) were used in the models. For each model we used two data sets, i.e. the Train set, encompassing about 80% and the Test set encompassing the remaining 20%. Models were developed as follows: binomial and multinomial predictions of steroid resistance (SR vs. SS; SR vs. SD vs. PSS, respectively), binomial prediction of steroid dependence (SD vs. PSS) and binomial prediction of susceptibility to nephrotic syndrome (NS vs. Controls). For the best performance, each model was characterized by and run under different hyperparameters and parameters, e.g. 50, 200, 100 and 50 neurons in the hidden layer (respectively for each prediction) and a tenfold cross-validation and 10
epochs for all Additional file 2: Table S2. The predictions were described by the sensitivity, specificity and LogLoss value for multinomials models with addition of area under the curve (AUC) value for binomials models. The importance of each predictor within a model was shown as percentage value.

Results
Demographic and clinical characteristics
There were 66 (53%) males and 58 (47%) females with the male to female ratio 1.2:1 in the patients group, and 31 (56%) males and 24 (44%) females with the male to female ratio 1.2:1 in the control group. The age of the NS onset (AOO) was available for all the patients in this study and categorized according to Sen et al. [7]: congenital (n = 2), infantile (n = 1), childhood (n = 107) and juvenile (n = 14). The mean AOO is shown in Table 1 and it did not differ when referred to NS subgroups (p = 0.3687), sex or histological findings (not shown, ns). 46 (37%) patients were not submitted to biopsy. Renal biopsy results were as follows: 16 (13%) FSGS, 25 (20%) MPGN (including mesangial proliferative glomerulonephritis with or without thickening of glomerular basement membrane) and 37 (30%) minimal change disease (MCD). FSGS was observed more frequently in SR group in comparison to other subgroups, although the result was only significant when compared to SS patients (p = 0.0263). No differences were found between histological outcomes when referred to sex or AOO (ns).

In total of 72 (58%) NS patients were observed adverse effects of glucocorticoid administration, e.g. 33 had osteoarthritis (26.6%), 11—obesity (9%), 9—growth deficit (7.3%), 8—steroid toxicity features on a face (6.5%),

| Parameters | NS | SR | SS | SD | PSS | C |
|------------|----|----|----|----|-----|---|
| Demographics | n = 124 | n = 53 | n = 71 | n = 31 | n = 40 | n = 55 |
| Male (%) | 66 (53.2%) | 36(56.4%) | 14 (45.2%) | 22 (55%) | 31 (56.4%) | 31 (56.4%) |
| Female (%) | 58 (46.8%) | 35 (49.3%) | 17 (45.2%) | 18 (45%) | 24 (43.6%) | 24 (43.6%) |
| AOO [years] | | | | | | 0.0263 |
| Histology | | | | | | 0.0367 |
| FSGS | 16 (13%) | 13 (24%) | 3 (4%) | 3 (10%) | 0 (0%) | 0 (0%) |
| MPGN | 25 (20%) | 12 (17%) | 9 (29%) | 3 (7.5%) | 3 (7.5%) | 3 (7.5%) |
| MCD | 37 (30%) | 20 (28%) | 10 (32%) | 10 (25%) | 27 (67.5%) | 27 (67.5%) |
| NA | 46 (37%) | 36 (51%) | 9 (29%) | 27 (67.5%) | 27 (67.5%) | 27 (67.5%) |
| Baseline characteristics: | | | | | | 0.0095** |
| Creatinine [mg/dl] | n = 111 | n = 43 | n = 68 | n = 31 | n = 37 | 0.0095** |
| Urea [mg/dl] | n = 110 | n = 42 | n = 68 | n = 31 | n = 37 | 0.2898 |
| Uric acid [mg/dl] | n = 100 | n = 41 | n = 59 | n = 25 | n = 34 | 0.4318 |
| Cystatin C [mg/l] | n = 66 | n = 24 | n = 42 | n = 15 | n = 27 | 0.7795 |
| eGFR [ml/min/1,73m3] | n = 111 | n = 44 | n = 67 | n = 29 | n = 38 | 0.0009*** |

Table 1 and it did not differ when referred to NS subgroups (p = 0.3687), sex or histological findings (not shown, ns). 46 (37%) patients were not submitted to biopsy. Renal biopsy results were as follows: 16 (13%) FSGS, 25 (20%) MPGN (including mesangial proliferative glomerulonephritis with or without thickening of glomerular basement membrane) and 37 (30%) minimal change disease (MCD). FSGS was observed more frequently in SR group in comparison to other subgroups, although the result was only significant when compared to SS patients (p = 0.0263). No differences were found between histological outcomes when referred to sex or AOO (ns).

In total of 72 (58%) NS patients were observed adverse effects of glucocorticoid administration, e.g. 33 had osteoarthritis (26.6%), 11—obesity (9%), 9—growth deficit (7.3%), 8—steroid toxicity features on a face (6.5%),

| Parameters | Single comparison p-value | Multiple comparison OR[95% CI]/ p-value |
|------------|---------------------------|---------------------------------|
| NS vs SR | p = 0.0218* | SR vs PSS: p = 0.0131* |
| NS vs SS | p = 0.03573 | SD vs PSS: p = 0.6836 |
| NS vs SD | p = 0.7795 | |
| NS vs PSS | p = 0.0141* | SR vs SS: p = 0.0141* |
| NS vs SS | p = 0.0263** | SR vs PSS: p = 0.0004** |
| NS vs SD | p = 0.093 | SD vs PSS: p = 0.0141* |

Table 1 Demographics and baseline laboratory characteristics of individuals used in this study

* Demographic data was analyzed by the t-student test and the laboratory data was analyzed using ANOVA with Holm adjustment (when data was normally distributed) and Dunn method with Benjamini–Hochberg adjustment and false discovery rate procedure (when data was not normally distributed). Post-hoc analysis was evaluated when global p-value reached significance, i.e. p ≤ 0.05 and significant results are shown in bold: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

NS, nephrotic syndrome; SR, steroid resistant; SS, steroid sensitive; SD, steroid dependent; PSS, primarily steroid sensitive; MCD, minimal change disease; MPGN, mesangial proliferative glomerulonephritis; FSGS, focal segmental glomerulosclerosis; AOO, age of onset; NA, not available, due to the biopsy not proceeded.
5—aggressive behavior and mood swings (4%) and 4—increased body hair (3.2%). 36 of those individuals (50%) were SR patients and in each subgroup more males than females had side effects, although not significantly.

Some patients had incomplete records at the time of diagnosis, therefore the total number of baseline laboratory characteristics is distinct for the subgroups (Table 1). The mean serum creatinine (s-creatinine) level differed significantly between SR and PSS (p = 0.0131), and SR and SS (p = 0.0218), while there was no significance between other subgroups. Also significantly different were eGFR values, not only for comparison of SR vs. PSS patients (p = 0.0014) and SR vs. SS (p = 0.0141), but also between SD and PSS patients (p = 0.0141). When histological findings were considered we spotted significant differences in s-creatinine levels for MCD vs. MPGN and FSGS patients (p = 0.005 for both), as well as in eGFR (p = 0.0106 and p = 0.0074, respectively) (ns). No significance for serum levels of uric acid, urea and cystatin C were observed. One PSS female patient presented G5 stage of kidney disease based on the eGFR measure (8.2 mg/dl), therefore the individual was excluded from these comparisons.

**Methylation status**

Analysis of SOCS3.2 promoter fragment revealed the full unmethylation pattern to be 15-fold more frequent in SR patients when compared both to overall SS and PSS as well as to SD patients (for all comparisons p < 0.0001) (Table 2). There were no differences in SOCS3.1 fragment methylation between the subgroups. The methylation patterns were not associated with patients’ sex or AOO (ns).

**Genetic variables**

The OR values for the frequencies of genotypes and alleles are shown in Additional file 3: Table S3. We detected heterozygous mutation in *NPHS2* (rs1057516414) in 6 (4.8%) NS patients as well as in 5 (9.1%) controls. Also, 1 heterozygous mutation in *EMP2* (rs587777481) was found in 1 (1.8%) control. Among 10 studied SNPs, 1 (rs139994842) showed a wild GG homozygote in all individuals in the study and was excluded from further statistical analyses. There were no significant differences in allele/genotype frequencies regarding patients’ sex (ns). In case of 4 SNPs (rs1922240, rs2070767, rs37972, rs4431401) we spotted significant differences in allele and genotype distribution in comparison to 1000 Genomes data (CEU population) (ns). Significant differences were observed between SR and SS patients in all three *ABCB1* variants for wild homozygotes (OR = 2.5, p = 0.0308 for rs1922240_AA; OR = 2.8, p = 0.0179 for rs1045642_AA; OR = 2.5, p = 0.0281 for rs2235048_CC). The Cochran-Armitage test and the test of deviation from additivity Additional file 4: Table S4 showed that those variants were significant under the dominant models. Similar, although insignificant, OR values were spotted for SR vs. PSS comparison. Interestingly, when SR and PSS, and not SS, groups were analyzed separately, pairwise LD analysis gave distinct association pattern for rs1922240_rs1045642, and rs1922240_rs2235048, while rs1045642_rs2235048 were in very strong LD in both groups (Fig. 2). When PSS and SD patients were compared, associated with steroid dependence were the

### Table 2 Methylation status of SOCS3 promoter fragments

| SOCS3 promoter fragment | Study Group | MM<sup>a</sup> n (%) | MU<sup>b</sup> n (%) | UU<sup>c</sup> n (%) | OR [95% CI] | P-value |
|-------------------------|-------------|------------------------|---------------------|----------------------|---------------------------------|---------|
| SOCS3<sub>1</sub>       | NS patients n = 124 | 3 (2%) | 115 (93%) | 6 (5%) | NS vs. C: 2.8 [0.3–23.4] | 0.3552 |
| <small>(− 1070/− 926 bp)</small> | | | | | | |
| SR patients n = 53 | 0 (0%) | 51 (96%) | 2 (4%) | SR vs. SS: 0.7 [0.1–3.7] | 0.6351 |
| SS patients n = 71 | 3 (4%) | 64 (90%) | 4 (6%) | SR vs. SD: 0.6 [0.1–4.3] | 0.5824 |
| PSS patients n = 40 | 1 (2%) | 37 (93%) | 2 (5%) | SR vs. PSS: 0.8 [0.1–5.5] | 0.7736 |
| SD patients n = 31 | 2 (6,5%) | 27 (87%) | 2 (6,5%) | SD vs. PSS: 1.3 [0.2–9.9] | 0.793 |
| Controls n = 55 | 1 (2%) | 53 (96%) | 1 (2%) | SS vs. C: 3.2 [0.4–29.7] | 0.3015 |
| SOCS3<sub>2</sub>       | NS patients n = 124 | 0 (0%) | 84 (68%) | 40 (32%) | NS vs. C: 6.1 [2.1–18] | 0.0011** |
| <small>(− 256/− 285 bp)</small> | | | | | | |
| SR patients n = 53 | 0 (0%) | 20 (38%) | 33 (62%) | SR vs. SS: 15.1 [5.8–39.3] | <0.0001*** |
| SS patients n = 71 | 0 (0%) | 64 (90%) | 7 (10%) | SR vs. SD: 15.4 [5.1–57.3] | <0.0001*** |
| PSS patients n = 40 | 0 (0%) | 36 (90%) | 4 (10%) | SR vs. PSS: 14.9 [4.6–48] | <0.0001*** |
| SD patients n = 31 | 0 (0%) | 28 (90.3%) | 3 (9.7%) | SD vs. PSS: 1 [0.2–4.7] | 0.9639 |
| Controls n = 55 | 0 (0%) | 51 (92.7%) | 4 (7.3%) | SS vs. C: 1.4 [0.4–5] | 0.6113 |

<sup>a</sup> Refers to ATG triplet; <sup>b</sup> MM refers to full methylation of the fragment; <sup>c</sup> MU refers to partial methylation of the fragment; <sup>d</sup> UU refers to full unmethylation of the fragment; significant results are shown in bold; *p < 0.05, **p < 0.01, ***p < 0.001

NS nephrotic syndrome; SR, steroid resistant; SS, steroid sensitive; SD, steroid dependent; PSS, primarily steroid sensitive
rare rs1045642_G variant under the additive (OR = 5.1, p = 0.007) and allelic (OR = 2.1, p = 0.0313) models, and the rare rs2235048_T variant under the dominant model (OR = 4.4, p = 0.0138), with comparable logistic regression results for both variants. In addition, rs1922240_G was more frequently observed in NS patients than in controls (p = 0.0318) and gave significant results in Cochran-Armitage test showing the strongest association in the additive model. Haplotype analysis (Table 3) revealed ABCB1_GGT to be associated with steroid sensitivity (p = 0.0106 for SR vs. SS; OR = 0.4, p = 0.0093 for SR vs. PSS), while AGT haplotype was associated with steroid dependent (OR = 4.2, p = 0.0028) and resistant (OR = 3.3, p = 0.0111) subphenotypes. CD73 rs9444348 and rs4431401 were in strong significant LD in all groups (Fig. 2). The rare rs9444348_A and AA were more frequently observed in SR group when compared to SS group (OR = 1.7, p = 0.0401; OR = 2.3, p = 0.0492, respectively), but also to PSS group (OR = 1.7, p = 0.0815; OR = 2.9, p = 0.0433, respectively), and logistic regression showed an association of rs9444348_A with steroid resistance under the additive model. CD73_AG haplotype was found to be associated with steroid resistance, however, the comparison only for SR and SS patients was significant (p = 0.0462). GG haplotype was significantly associated with steroid sensitivity, since it was absent in SR group. The rest of the tested SNPs gave insignificant results. The most confusing variant in the study was MIF CNV polymorphism rs5844572 and only some of the results were significant Additional file 3: Table S3. Interestingly, we spotted differences in allele/genotype and haplotype frequencies between the histological findings. The wild rs1922240_A was associated with MPGN when compared both to MCD (OR = 2.5, p = 0.0197) and FSGS (OR = 2.6, p = 0.0385) (ns). In addition, though no differences were seen for rs1045642 and rs2235048 in single locus analysis, ABCB1_AGT haplotype was significantly associated with MPGN (p = 0.0374) (Additional file 5: Table S5). Also, the rare rs2070767_A was almost threefold more frequent (p = 0.0101) and AA homozygote was almost sevenfold more frequent (p = 0.0945) in patients with MPGN when compared to MCD. On the other hand, with MCD were associated the wild rs2070767_G (p = 0.0101) and rs2070767_GG (p = 0.0222) when compared to MPGN, but not FSGS. We spotted high OR value for MIF_5AG haplotype in FSGS patients in comparison with MCD but not MPGN patients, therefore it might be the result of the differences in the groups' size, since the haplotype frequency was 'zero' in both latter groups.

Prediction modeling
In total, 4 prediction models were developed and the results are presented in Table 4.

As s-creatinine levels differed significantly between patients in this study it was added as a predictor into models predicting the NS subtypes. eGFR was omitted as it was a derivative of the s-creatinine value. Steroid resistance prediction reached 92% (area under the curve, AUC = 0.778) in binomial and 63% in multinomial calculations. The majority of test phenotypes in binomial prediction were ascribed to sensitive outcome giving low SR sensitivity (33%) most probably due to a very limited number of SR vs. SS patients in the Test set (3 vs. 21). Steroid dependence was predicted with overall capacity of 80% (AUC = 0.720) in binomial, and with sensitivity of 67% in multinomial model. The importance of each marker in the models is presented in Additional file 6: Table S6.

Discussion
Prediction models have previously been assessed in medical forecasting in various conditions, including renal disorders, e.g. MCD, IgA nephropathy and progression to chronic kidney disease using both categorical and
| Gene | SNPs | Haplotype | Frequency | SR vs. SS | SR vs. PSS | SR vs. SD | SD vs. PSS | NS vs. C |
|------|------|-----------|-----------|-----------|-----------|-----------|------------|----------|
|      |      |           |           | NS SR SS SD PSS C OR [CI 95%] p-value | OR [CI 95%] p-value | OR [CI 95%] p-value | OR [CI 95%] p-value | OR [CI 95%] p-value |
| ABCB1 | rs1922240 | AAC | 0.33 | 0.381 0.305 0.19 0.398 0.43 | 1.4 [0.8–2.4] 0.2102 | 0.9 [0.5–1.7] 0.816 | 2.7 [1.3–5.6] **0.0098** | 0.4 [0.2–0.8] 0.0077 |
|      | rs1045642 | AGT | 0.167 | 0.213 0.16 0.262 0.077 0.224 | 1.4 [0.7–2.7] 0.2818 | 3.3 [1.3–8.4] *0.0111 | 0.8 [0.4–1.6] 0.4723 | 4.2 [1.6–11.5] **0.0028** |
|      | rs2235048 | GAC | 0.174 | 0.185 0.153 0.165 0.14 0.143 | 1.2 [0.6–2.4] 0.5004 | 1.4 [0.6–3.2] 0.4102 | 1.2 [0.5–2.7] 0.7432 | 1.2 [0.5–3.1] 0.676 |
|      | GGT | 0.029 | 0.211 0.361 0.335 0.385 0.203 | 0.5 [0.3–0.8] *0.0106 | 0.4 [0.2–0.8] **0.0093** | 0.5 [0.3–1.1] 0.764 | 0.8 [0.4–1.6] 0.5364 | 1.7 [1–2.9] 0.0742 |
|      | AGC | 0.016 | 0 0.001 0.048 0 0 | 0.001 [0–0.2] 0.2186 | – – 0 [0–0.01] *0.0347 | – 0.0468 | – – – 0.186 |
|      | GGC | 0 0.009 0 0 0 0 | 18.5 [6.1–3605] 0.3602 | – 0.3843 | – 0.4436 | – – – – |
|      | CD73 | rs9444348 | AG | 0.524 | 0.594 0.472 0.468 0.475 0.464 | 1.7 [1–2.8] *0.0462 | 1.7 [0.9–3] 1.058 | 1.7 [0.9–3.2] 0.097 | 1.7 [0.9–3.2] 0.097 |
|      | rs4431401 | GA | 0.447 | 0.396 0.486 0.484 0.487 0.473 | 0.7 [0.4–1.2] 0.1797 | 0.7 [0.4–1.3] 0.214 | 0.7 [0.4–1.3] 0.2904 | 0.5 [0.3–1.1] 0.764 |
|      | GG | 0.024 | 0 0.042 0.048 0.038 0.064 | – 0.0322 | – 0.0445 | – 0.0223 | 1.3 [0.3–6.7] 0.7491 | 0.4 [0.1–1.1] 0.0668 |
|      | AA | 0.004 | 0.009 0 0 0 0 | – 0.2462 | – 0.3838 | – 0.4431 | – – – 0.499 |
|      | MIF | rs5844572 | SAG | 0 0 0 0.017 0 0 | – – – 0.01 [0–0.3] | 0.2553 | 753.4 [307.7–18,466.1] | 0.3534 | – – |
|      | rs2070767 | SAT | 0.266 | 0.264 0.267 0.273 0.25 0.236 | 1 [0.6–1.8] 0.9877 | 1.1 [0.6–2.1] 0.787 | 1 [0.5–2] 0.9294 | 1.2 [0.5–2.5] 0.7077 | 1.1 [0.7–1.9] 0.6302 |
non-categorical variables [15–17]. Depending on an algorithm and machine learning technique used, more robust and more quickly obtained results are being achieved. However, most of them apply to case–control studies rather than prediction of drug response or secondary subphenotype under the same condition. Here, we present the results of analysis of several variables and their ability to predict type of steroid response in children with nephrotic syndrome using Neural Network—a method that have been successfully used in medical diagnosis of e.g. Huntington disease [18], osteoporosis [19], the prediction of cardiovascular autonomic dysfunction [20] or patients prognosis depending on cancer subtypes and gene mutations [21]. All variables used in this study were chosen based on our previous experience and their proved/suspected role in other populations.

In this study, we achieved accuracy of 92% and 63% for steroid resistance prediction in NS for binomial and multinomial calculations, respectively. The strongest prediction marker was methylation status of SOCS3.2 fragment, which was confirmed by all other statistical tests performed. Previously we showed full unmethylation of the same promoter fragment with probable correlation with SOCS3 upregulation in Polish SRNS children [22]. Here, the results were consistent, showing about 15-fold higher frequency of unmethylated SOCS3 promoter in steroid resistant group when compared to overall steroid sensitive, but also to primarily sensitive and dependent, groups. It therefore reaffirms the hypothesis of epigenetic regulation mechanism of SOCS3 expression in steroid resistance in the course of NS in Polish children and is worth examining in other populations.

The strongest genetic marker turned out to be ABCB1. It encodes multidrug resistant protein which polymorphic variants have been linked to decrease in drug’s accumulation in the cell. Here, the wild rs1045642_A variant correlated with steroid resistance which is comparable with most studies about steroid unresponsiveness in nephrotic patients of different ethnics [23–26]. The A allele also correlated with increased kidney graft failure [27] and development of interstitial fibrosis and tubular atrophy in kidney grafts [28], whereas the G allele lowered the risk of post kidney transplant complications [29]. Only one study showed that African-American and CEU rs1045642_A-carriers were better steroid responders than the G-carriers after a heart transplant [30]. Worth mentioning, the frequency of rs1045642 alleles in general Polish population is quite distinct among the studies [23, 31, 32], which might be the result of subregions within the country that were taken under consideration. Notably, all three ABCB1
variants studied here were significantly differently distributed when compared to CEU population data, which suggests favorable trend for studying variants with presumable clinical correlations in highly geographically homogeneous groups like in this study.

Little is known about $ABCB1$ rs2235048. Its wild C variant was previously linked to poorer response in schizophrenia and [33], consistently, here rs2235048_C correlated with poorer response to steroids, however it might be an indirect association as rs2235048 is an intron variant and it was in very strong LD with rs1045642. On the contrary, no association has been reported so far for rs1922240. Here, the rare G allele was associated with nephrotic syndrome occurrence, whereas wild A and AA—with MPGN when compared to FSGS or MCD. It is an intriguing finding, since both FSGS and MPGN were equally distributed in our steroid resistant patients, and it is FSGS that is most often assigned to steroid resistant nephrotic syndrome. In fact, Chanchlani et al.[34] stated that researchers falsely tend to combine FSGS and SR phenotypes under one category, ironically explaining the discrepancies in the results by the differences in definitions, clinical management and ethnic component among the studies. Indeed, that was acknowledged by many other authors [1, 4, 35–38]. Here, each of the three variants explained about 7% of the trait and the most detrimental $ABCB1$ haplotype was AGT, being associated with steroid resistance and steroid dependence (Table 3), and, independently, with mesangial proliferative changes (Additional file 5: Table S5).

We spotted promising results also for rs9444348 and rs4431401 in $CD73$, a targeted molecule of miR-30a and surface marker of mesenchymal stem cells as potential indicators of an early-stage renal damages in chronic kidney disease [39]. MiR-30a upregulation has been previously observed in urine of FSGS patients, while its downregulation was associated with steroid sensitivity in NS [40] Here, the rare alleles of both SNPs were associated with steroid resistance, therefore $CD73_{AG}$ haplotype was a risk factor for developing steroid unresponsiveness. Best of our knowledge, both variants have been, so far, examined in two research [39, 41], one of which concerned Chinese NS patients.28 Interestingly, the study presented $CD73_{AG}$ haplotype as protective against nephrotic syndrome. However, consistently with their findings, rs3124591 in the other targeted molecule of miR-30a—$NOTCH1$, showed no association with NS or steroid subtypes in our study. Nevertheless, it is difficult to compare such results as the authors did not refer to the subtypes of drug response and because of ethnic differences [39, 40].

Promising, yet the most inconclusive in this study were $MIF$ variants. The rare, high-expression rs5844572_CATT7 allele was shown to be associated with severe forms of steroid resistance in the course of Japanese ulcerative colitis patients [42], with increased $MIF$ expression in more severe forms of glomerulonephritis [43], and with early onset of rheumatoid arthritis [44], while the wild, low-expression CATT5 allele correlated with milder forms of a disease in the latter studies. Consistent, here, CATT7 and CATT77 were more frequent in detrimental unmethylated pattern of SOCS3.2 fragment and over twofold more frequent in NS patients when compared to controls, while CATT5 and CATT55 were associated more with partial methylation in steroid sensitive patients, although only some of the result were significant (ns). Interestingly, rs5844572 reached 11–14% of importance in both NS and SR prediction models. Other $MIF$ variant, the rare rs2070767_A, was significantly linked to MPGN lesion while the wild G was associated with MCD (ns). Little is known about true association of both SNPs with susceptibility to a trait and only Gao et al.[45] demonstrated rare rs2070767_A as a risk factor for higher $MIF$ expression in acute lung injury in African-Americans.
The most dissatisfying results in this study were observed for GLCCI1, which expression was previously shown to be induced directly by the steroids and impaired by rs37972 which correlated with poorer response to inhaled steroids in asthmatic non-Hispanic patients, as well as poorer response to steroids and activity of the disease in Netherland rheumatoid arthritis patients [24, 46–48]. In our Polish patients rs37972 alleles were equally distributed within all individuals, regardless the disease, its subtypes, age of onset, biochemical or histological parameters.

Out of over 50 genes and their nucleotide variants associated with SRNS, in only one, i.e. EMP2, single nucleotide variants have been assigned both to steroid resistance (rs587777482) and steroid sensitivity (rs730882194 and rs587777481) in Turkish NS children [49]. Interestingly, here, only rs587777481, which is a truncating mutation, was only present as a heterozygote in 1 (0.02%) control. Also, no m.3243A>G (rs199474657) in MT-TL1, previously linked to kidney failure, FSGS and SRNS [50, 51], was found in this study. Next, we analyzed NPHS2 mutations that have been assigned to 30% of steroid resistant forms of NS in children, especially R138Q (rs574315342) and R229Q (rs1057516414), commonly attributed to SRNS in East Europeans [2, 4, 52]. We spotted no R138Q, whereas 6 patients (3 SR, 2 PSS, 1 SD) and 5 controls had heterozygous R229Q. Although we do not know the steroid responsiveness status of those controls, we were not able to verify mutation’ prediction capability, as its alleles number did not reach a threshold within subgroups (n = 5). Interestingly, Caridi et al. [53] showed that single heterozygous R229Q is not sufficient for SRNS diagnosis and others demonstrated heterozygous R229Q to be a common variant present in 3% of the general European population [54, 55]. Additionally, two patients with age of onset at 2 and 3 months, respectively, were suspected of congenital NS and were subjected to sequencing of coding and non-coding regions of NPHS2, however, no known or novel mutations were spotted (ns).

When laboratory parameters were considered, we observed that s-creatinine level was significantly higher in steroid resistant in comparison with steroid sensitive and primarily sensitive patients. The well-known association of higher s-creatinine level with steroid resistance is most probably due to its role in progression to end stage renal disease and long-term prognosis in NS, which is generally poorer for unresponsive patients [35, 56, 57]. Worth mentioning, mean s-creatinine and eGFR differed significantly between MCD and other histopathological findings in this study, though MCD was a dominant lesion regardless the steroid response. Zhu et al. [17] demonstrated lower s-creatinine levels in MCD vs. other kidney diseases in Chinese patients, however it did not have enough diagnostic value in MCD risk model. Other serum parameters commonly used to monitor progression of renal disorders, i.e. urea, uric acid and cystatin C, did not differ when referred to our steroid subgroups, sex or histological lesions. Lately, only one study [56], has reported that different serum urea levels were able to distinguish steroid responsive and unresponsive Turkish NS patients. Still, most studies focus on differentiating NS (or other disease) subjects from controls, rather than on secondary features, e.g. drugs unresponsiveness [10, 57, 58]. Out of other demographic variables, i.e. male sex and younger age of onset, commonly ascribed to steroid unresponders [3, 24, 25], we did not observe such association, which was in agreement with others [56, 59]. Nevertheless, the results are difficult to compare due to differences in the individuals’ number and ethnic origin among the studies.

Despite the promising results in the steroid resistance area, equally strong value of this study is 60–67% models’ capacity of predicting steroid dependence in NS. We show an association of even single ABCB1 rare rs1045642_G allele and rare rs2235048_T with SD outcome, which has scarcely been studied elsewhere mainly due to combining steroid dependent and primarily sensitive into one category. One study correlated steroid dependence in Egyptian NS patients with young age of onset, male sex and late responders, however these were not very specific markers and the number of individuals tested were quite small (n = 24) [59].

Conclusions

We demonstrated significant association of rs1922240, rs1045642 and rs2235048 in ABCB1 and rs9444348 and rs4431401 in CD73, along with serum creatinine level and unmethylation of a fragment of SOCS3 promoter, with steroid resistance in a cohort of Polish children with nephrotic syndrome, that comprised SR prediction model. The results of MIF CNV were ambiguous, yet worth analyzing in a bigger cohort. The number of individuals were the biggest limitation of this study and a bigger cohort would definitely be needed for replicate studies. Definitely the strong value of our work is an association of ABCB1 rs1045642 and rs2235048 with steroid dependent outcome in NS and it is worth analyzing both in bigger cohort, also one of other ethnicities. Next, worth mentioning is an association of CD73 rs9444348 and rs4431401 and MIF rs2070767 with histopathological lesions regardless the steroid response, which has previously been suggested, but not confirmed [5]. Lastly,
our study supports the view that highly heterogeneous disease such as nephrotic syndrome and its multiple response-to-drug outcomes should be studied in as much as possible homogeneous cohorts.

**Abbreviations**

AOO: Age of onset; C-A: Cochran Armitage trend test; CNV: Copy number variant; eGFR: Estimated glomerular filtration rate; FSGS: Focal segmental glomerulosclerosis; ISKDC: International Study of Kidney Disease in Children; LD: Linkage disequilibrium; MCD: Minimal change disease; MPGN: Mesangial proliferative glomerulonephritis; MCD: Minimal change disease; NN: Neural network; NS: Nephrotic syndrome; PSS: Primarily steroid sensitive; SD: Steroid dependent; SNP: Single nucleotide polymorphism; SR: Steroid resistant; SS: Steroid sensitive.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12967-021-02790-w.

Additional file 1: Table S1. Characteristics of 16 single nucleotide variants analyzed in this study. The length of the t-tail of single base extension (sbe) primers is shown as numbers in parentheses. SNP information was retrieved from Ensembl Genome Browser. All genes' accession numbers were described using GenBank database, and variants' rs numbers were verified using Variant Validator.

Additional file 2: Table S2. Parameters and hyperparameters for Neural Network prediction modeling. Hyperparameters were used for searching for the best architecture of the network, while parameters are those, under which the designed models were run.

Additional file 3: Table S3. Genotype and allele frequencies. All frequencies were calculated for 9 distinct SNPs and 1 CNV and the association with nephrotic syndrome phenotypes upon steroid treatment was determined using the Fisher's exact test. Calculations were made for dominant (AA vs. Aa + aa), recessive (AA + Aa vs. aa), ever-dominant (Aa vs. AA + aa) and allelic (A vs. a) genetic models. Significant results are shown in bold. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Abbreviations: NS, nephrotic syndrome; SR, steroid resistant; SS, steroid sensitive; SD, steroid dependent; PSS, primarily steroid sensitive.

Additional file 4: Table S4. Logistic regression analysis followed by testing deviation from additivity in a multiplicative model. Logistic regression was applied for additive, dominant, recessive, allelic and genotypic models. The regression coefficients represented as chi square values indicates the increase of the effect of each minor allele in creating a phenotype. NA is displayed when the number of rare genotypes in at least one of the subgroups is less than the default value, i.e. 10. Significant results are shown in bold. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. † Multiplicative model testing deviation from additivity; ‡ Genotypic model testing deviation from additivity; Abbreviations: NS, nephrotic syndrome; SR, steroid resistant; SS, steroid sensitive; SD, steroid dependent; PSS, primarily steroid sensitive.

Additional file 5: Table S5. Haplotype frequencies and association with histopathological findings in NS patients. † The p-value number in a column for a haplotype is a global p-value. S, 6 and 7 numbers in MIF haplotypes refer to the number of CATT repeats. Lowest frequency threshold was set to 0.009. Significant results are shown in bold. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Abbreviations: MCD, minimal change disease; MPGN, mesangial proliferative glomerulonephritis; FSGS, focal segmental glomerulosclerosis; NA, not available, due to the biopsy not proceeded.

Additional file 6: Table S6. Importance of studied variables for Neural Network. The importance was shown as percentage values (both for binomial and multinomial comparisons). Abbreviations: NS, nephrotic syndrome; SR, steroid resistant; SS, steroid sensitive; SD, steroid dependent; PSS, primarily steroid sensitive; AOO, age of onset; s-creatinine, serum creatinine.

**Acknowledgements**

The authors thank Poznan Supercomputing and Networking Center (Project ID: 416).

**Authors’ contributions**

KZ: Conceptualization, Methodology, Data curation, Writing. PZ: Conceptualization, Software, Formal analysis, Writing. MS: Methodology, DO-N: Resources, Reviewing and Editing. JZ: Resources, Reviewing and Editing. MN: Supervision, Visualization. All authors read and approved the final manuscript.

**Funding**

This work was financially supported by the Poznan University of Medical Sciences grant for Young Scientists No. 502-14-02229373-09513.

**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Written informed consents were obtained from all patients’ guardians. All procedures performed with human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration, its later amendments and ethical standards. All study procedures were approved by the Ethics Committee of Poznan University of Medical Sciences.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1 Department of Histology and Embryology, University of Medical Sciences, Swożewickiego St 6, 60-781 Poznan, Poland. 2 Institute of Bioorganic Chemistry, Department of RNA Metabolism, Polish Academy of Sciences, Zygmunta Noskowskiego St 12/14, 61-704 Poznan, Poland. 3 Clinic of Pediatric Nephrology and Hypertension, University of Medical Sciences, Szpitalna St 27/33, 60-572 Poznan, Poland.

**Received:** 14 October 2020   **Accepted:** 15 March 2021

**Published online:** 30 March 2021

**References**

1. Anjilaje EA, Oluotla A. Prospects of genetic testing for steroid-resistant nephrotic syndrome in Nigerian children: a narrative review of challenges and opportunities. Int J Nephrol Renovascular Dis. 2019;12:119–36.

2. Nourbakhsh N, Mak RH. Steroid-resistant nephrotic syndrome: past and current perspectives. Pediatric Health Med Ther. 2017;8:29–37. https://doi.org/10.2147/PHMT.S100803.

3. Bennett MR, Piyanaphanee N, Czech K, Mitsnefes M, Devarajan P. NGAL distinguishes steroid sensitivity in idiopathic nephrotic syndrome. Pediatr Nephrol. 2012;27(5):807–90. https://doi.org/10.1007/s00467-011-2075-7.

4. Rood IM, Deegens JKL, Wetzels JFM. Genetic causes of focal segmental glomerulosclerosis: implications for clinical practice. Nephrol Dial Transplant. 2012;27:882–90. https://doi.org/10.1093/ndt/gfr771.

5. Lovric S, Ashraf S, Tan W, Hildebrandt F. Genetic testing in steroid-resistant nephrotic syndrome: when and how? Nephrol Dial Transplant. 2016;31:1802–13. https://doi.org/10.1093/ndt/gft355.

6. Trautmann A, Bodria M, Ozaltin F, Gheisari A, Melk A, Azocar M, et al. Spectrum of steroid-resistant and congenital nephrotic syndrome in children: the PodoNet registry cohort. Clin J Am Soc Nephrol. 2015;10:592–600. https://doi.org/10.2126/cjn.06260614.
7. Sen ES, Dean P, Yarram-Smith L, Bierzyńska A, Woodward G, Buxton G, et al. Clinical genetic testing using custom-designed steroid-resistant nephrotic syndrome gene panel: analysis and recommendations. J Med Genet. 2017;54:795–804. https://doi.org/10.1136/jmedgenet-2017-104811.

8. Zhang H, Wang Z, Dong LQ, Guo YN. Children with steroid-resistant nephrotic syndrome: long-term outcomes of sequential steroid therapy. Biomed Environ Sci. 2016;29(9):650–5. https://doi.org/10.3967/bes2016.087.

9. Zaworska K, Zawierucha P, Ostalska-Nowicka D, Nowicki M. SOCS3 is epigenetically up-regulated in steroid resistant nephritic children. Acta Biochim Pol. 2016;63(1):131–8. https://doi.org/10.18388/abp.2015.1105.

10. National Kidney Foundation. KDQQ clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am J Kidney Dis. 2002;39(suppl 1):51–266.

11. Aicken M, Gensler H. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm Methods. Am J Public Health. 1996;86(5):726–8. https://doi.org/10.2105/AJPH.86.5.726.

12. Benjamin Y, Hochberg Y. Controlling the false discovery rate: a practical example for multiple testing. J Roy Statist Soc Series B Methodol. 1995;57(1):289–300. https://doi.org/10.2307/2346101.

13. Bagos PG. Genetic model selection in genome-wide association studies: robust methods and the use of meta-analysis. Stat Appl Genet Mol Biol. 2013;12(3):289–308. https://doi.org/10.1515/sgmb-2012-0016.

14. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 2009;19(4):519–23. https://doi.org/10.1038/cr.2009.33.

15. Almansour NA, Syed HF, Khayat NR, Altheeb RK, Juri RE, Alhiyafi J, et al. Neural network and support vector machine for the prediction of chronic kidney disease: A comparative study. Comput Biol Med. 2019;109:101–11. https://doi.org/10.1016/j.compbiomed.2019.04.017.

16. Di Noia T, Ostuni VC, Pesce F, Binetti F, Naso D, Schena FP, et al. An end stage kidney disease predictor based on an artificial neural networks ensemble. Expert Syst Appl. 2013;40:4438–45. https://doi.org/10.1016/j.eswa.2013.01.046.

17. Zhu H, Han Q, Zhang D, Wang Y, Gao J, Geng W, et al. A diagnostic model for minimal change disease based on biological parameters. Peer J. 2018;6:e2437. https://doi.org/10.7717/peerj.2437.

18. Lauraitis A, Maskeliūnas R, Damaševičius R. ANN and fuzzy logic based modeling of steroid response in nephrotic syndrome children. BioMed Res Int. 2013;2013:280345. https://doi.org/10.1155/2013/280345.

19. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 2009;19(4):519–23. https://doi.org/10.1038/cr.2009.33.

20. Liu J, Tang ZH, Zeng F, Li Z, Zeng Z et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 2009;19(4):519–23. https://doi.org/10.1038/cr.2009.33.

21. Flaster H, Bernhagen J, Calandra T, Bucala R. The macrophage migration inhibitory factor (MIF): genetic evidence for participation in early onset and early course rheumatoid arthritis. Cytochrome. 2013;36(3):759–65. https://doi.org/10.1016/j.cytobio.2012.03.022.
45. Gao L, Flores C, Fan‑Ma S, Miller EJ, Motra J, Moreno L, et al. Macrophage migration inhibitory factor in acute lung injury: expression, biomarker and associations. Transl Res. 2007;150(1):18–29. https://doi.org/10.1016/j.trsl.2007.02.007.

46. Nishibori Y, Katayama K, Parikka M, Oddsson A, Nukui M, Hultenby K, et al. GLCCI1 deficiency leads to proteinuria. J Am Soc Nephrol. 2011;22:2037–46.

47. Tantisira KG, Lasky‑Su J, Harada M, Murphy A, Litonjua AA, Himes BE, et al. Genomwide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med. 2011;365(13):1173–83. https://doi.org/10.1056/nenglj20111063.

48. Quax RAM, Koper JW, Huisman AM, Weel A, Hazes JM, Lamberts SWJ, et al. Polymorphisms in the glucocorticoid receptor gene and in the glucocorticoid‑induced transcript 1 gene are associated with disease activity and response to glucocorticoid bridging therapy in rheumatoid arthritis. Rheumatol Int. 2015;35(8):1325–33.

49. Gee HY, Ashraf S, Wan X, Vega‑Warner V, Estève‑Rudd J, Lovric S, et al. Mutations in EMP2 cause childhood‑onset nephrotic syndrome. Am J Hum Genet. 2014;94(6):884–90. https://doi.org/10.1101/ajhg.2014.04.010.

50. Finsterer J. Kidney transplantation in m3243A>G carriers has outcome implications. Clin Kidney J. 2020. https://doi.org/10.1093/ckj/sfaa025.

51. Löwik MM, Hol FA, Steenbergen EJ, Wetzels JFM, van den Heuvel LPWJ. Mitochondrial tRNALeu(UUR) mutation in a patient with steroid resistant nephrotic syndrome and focal segmental glomerulosclerosis. Nephrol Dial Transplant. 2005;20(2):336–41. https://doi.org/10.1093/ndt/gfh546.

52. Lipiska BS, Balasz‑Chmielewska I, Morzuch L, Wasielewski K, Vetter D, Borzecka H, et al. Mutational analysis in podocin‑associated hereditary nephrotic syndrome in Polish patients: founder effect in the Kashubian population. J Appl Genetics. 2013;54:327–33. https://doi.org/10.1007/s13353‑013‑0147‑z.

53. Caridi G, Gigante M, Ravani P, Trivelli A, Barbano G, Scalfi F, et al. Clinical features and long‑term outcome of nephrotic syndrome associated with heterozygous NPHS1 and NPHS2 mutations. Clin J Am Soc Nephrol. 2009;4(6):1065–72. https://doi.org/10.2215/cjn.09100808.

54. Pereira AC, Pereira AB, Mota GF, Cunha RS, Herkenhoff FL, Pollak MR, et al. NPHS2 R229Q functional variant is associated with microalbuminuria in the general population. Kidney Int. 2004;65(3):1026–30. https://doi.org/10.1111/j.1523‑1755.2004.00479.x.

55. Franceschini N, North KE, Kopp JB, McKenzie L, Winkler C. NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: a HuGE review. Genet Med. 2006;8(2):63–75. https://doi.org/10.1097/01.gim.0000200947.09626.1c.

56. Kara A, Gurogoz MK, Kara M, Aydin M. Evaluation of genetic polymorphisms for determining steroid response in nephrotic children. Ann Clin Lab Sci. 2018;48(4):478–83.

57. Gooding JR, Agrawal S, McRitchie S, Acuff Z, Merchant ML, Klein JB, et al. Predicting and defining steroid resistance in pediatric nephrotic syndrome using plasma metabolomics. Kidney Int Rep. 2019;5(1):81–93. https://doi.org/10.1016/j.ekiri.2019.09.010.

58. Sampson AL, Singer RF, Walters GD. Unc‑acid lowering therapies for preventing or delaying the progression of chronic kidney disease. Cochrane Database Syst Rev. 2017;10(10):CD009460. https://doi.org/10.1002/14651858.cd009460.pub2.

59. Abdel‑Hafez MA, Abou‑El‑Hana NM, Erfan AA, El‑Gamasy M, Abdel‑Nabi H. Predictive risk factors of steroid dependent nephrotic syndrome in children. J Nephropathol. 2017;6(3):180–6. https://doi.org/10.15171/jnp.2017.31.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.