# Genetic and *in vivo* determinants of glucocorticoid sensitivity in relation to clinical outcome of childhood nephrotic syndrome

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**Following initial glucocorticoid treatment, the clinical course in children with nephrotic syndrome is highly variable. Intrinsic sensitivity to glucocorticoids might be a determinant of this variability. Functional polymorphisms of the glucocorticoid receptor gene *NR3C1* have been associated with either relatively impaired (GR-9β) or increased (*Bcl*I) glucocorticoid sensitivity. Here, in a prospective, well-defined cohort of children with nephrotic syndrome, we evaluated both carriage of GR-9β + TthIII-1 and *Bcl*I haplotypes in 113 children and a dexamethasone suppression test in 90 children in relation to their clinical outcome over a median follow-up of 4.4 years. Carriers of GR-9β + TthIII-1 had a significantly higher incidence of steroid dependence 13/25 (52%) compared with noncarriers 19/75 (25%) with a hazard ratio adjusted for gender, age, and descent of 3.04 with 95% confidence interval 1.37–6.74. Both first and frequent relapses happened significantly more often in GR-9β + TthIII-1 carriers than in noncarriers. There were no significant differences in therapeutic outcomes between carriers and noncarriers of the *Bcl*I haplotype. Results of the dexamethasone test showed no associations with clinical outcome. Thus, the GR-9β + TthIII-1 haplotype of the glucocorticoid receptor gene offers new insights into the clinical course of children with nephrotic syndrome.**

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Childhood nephrotic syndrome denotes a heterogenous group of glomerulopathies, characterized by heavy proteinuria and hypoalbuminemia, occurring in one to seven per 100,000 children each year. In Caucasians, around two-thirds of the patients are male and most are under 8 years of age.[1–5] Though relatively benign in terms of renal function, this disease has a high relapse rate, which puts children at risk of severe complications.[1,6] The pathophysiology has only partly been elucidated and is likely to be multifactorial.[7,8] A beneficial effect of several immune modulating agents was established.[3,9,10]

Prednisolone is used as first line treatment for childhood nephrotic syndrome, as it induces remission of proteinuria in 90–95% of patients.[11,12] Following initial prednisolone therapy, considerable variability exists in terms of relapses and side effects.[1,6,12] Previous efforts to explain the variability in clinical course of nephrotic syndrome in children have hardly been successful. Studies reporting prognostic value of baseline characteristics were mostly retrospective and their results contradictory.[13–19] Obtaining such factors within prospective settings has been problematic due to the relatively low incidence of this disease.

Part of the variability in the clinical course of childhood nephrotic syndrome may be explained by interindividual differences in glucocorticoid handling and metabolism. The complex mechanisms of glucocorticoid action give rise to heterogeneity of glucocorticoid sensitivity, which is known to exist in the general population.[20–22] Sensitivity to glucocorticoids depends on both functionality and expression of the intracellular glucocorticoid receptor,[23] which is present in virtually all body cells, including cells comprising the glomerular filtration barrier.[3]

Several functional single-nucleotide polymorphisms of the glucocorticoid receptor gene *NR3C1* have been identified.[23] Individuals harboring the minor alleles of the ER22/23EK

(rs6189 and rs6190) and/or GR-9β (rs6198) polymorphisms display relatively impaired glucocorticoid sensitivity.[24,25] The minor alleles of both the *Bcl*I (rs41423247) and the N363S (rs6195) polymorphisms are associated with increased glucocorticoid sensitivity.[26] The potential clinical relevance of these genetic variations is largely unknown for patients with nephrotic syndrome. Zalewski *et al.* associated a haplotype containing the *Bcl*I polymorphism with rapid remission (within 7 days) in children with nephrotic syndrome. Unfortunately, associations between this haplotype and other parameters of clinical outcome were not reported in this study.[27]

In addition to known genetic variations in the glucocorticoid gene, intrinsic sensitivity to glucocorticoids is reflected *in vivo* by means of hypothalamus–pituitary–adrenal axis function tests. Interindividual variations of this feedback response have been found after low dose dexamethasone challenges, under both physiological and pathophysiological conditions.[28–31] Impaired adrenal response has been previously associated with increased risk of relapse in patients with nephrotic syndrome. Yet, these studies focused on adrenal function during or immediately after exogenous glucocorticoid exposure,[32,33] not on the relationship between intrinsic variations in glucocorticoid sensitivity and clinical outcome.

The aim of the present study was to investigate whether glucocorticoid sensitivity is associated with therapeutic and side effects of prednisolone in childhood nephrotic syndrome. We were able to investigate this in a well-defined, prospective cohort of Dutch children diagnosed with nephrotic syndrome.[12]

## RESULTS

### Patient characteristics

Within the umbrella study cohort ($n = 150$), 113 children with nephrotic syndrome were genotyped and 90 children performed a very low dose dexamethasone suppression test. As explained in the methods section, children initially received prednisolone for either 3 months or 6 months. The prescribed cumulative dose of prednisolone was equal in both initial treatment regimens ($\sim 3360\ mg/m^2$), and the greater part was administered in the first 3 months. Baseline and clinical characteristics are shown in Table 1. The majority of patients had at least one relapse, approximately half of the children had frequent relapses and around one-third were steroid dependent. Time to the first relapse was shorter in children with frequent relapses compared with patients without frequent relapses, median (interquartile range (IQR)) 5 months (4–8) vs. 10 months (7–13), $P = 0.001$.

**Table 1 | Baseline and clinical outcome characteristics**

| | VLD-DST | GR SNPS |
|---|---|---|
| *n* | 90 | 108 |
| Number of males (%) | 58 (64) | 71 (66) |
| Age at onset, yrs, median (IQR) | 4.0 (3.1 to 6.5) | 4.1 (3.2 to 6.1) |
| *Descent, n (%)* | | |
| Western-European | 66 (73) | 76 (70) |
| Non-Western-European | 10 (11) | 14 (13) |
| Mixed | 8 (9) | 10 (9) |
| Unknown | 6 (7) | 8 (7) |
| *Initial prednisolone treatment, n (%)* | | |
| 3 months | 45 (50) | 61 (56) |
| 6 months | 45 (50) | 47 (43) |
| Duration of follow up, yrs, median (IQR) | 4.3 (3.1 to 5.0) | 4.4 (3.3 to 5.0) |
| *Therapeutic outcome* | | |
| Primary steroid resistance, *n* (%) | 2 (2) | 8 (7) |
| Time to first remission, days, median (IQR) | 10 (8 to 13) | 10 (8 to 14) |
| One or more relapses, *n* (%) | 67 (74) | 79 (73) |
| Total number of relapses, median (IQR) | 3 (1 to 5) | 3 (1 to 6) |
| Relapses per year of follow up, median (IQR) | 0.6 (0.2 to 1.2) | 0.75 (0.30 to 1.40) |
| Frequent relapses, *n* (%) | 44 (49) | 56 (52) |
| With steroid dependence, *n* (%) | 24 (27) | 32 (30) |
| *Adverse effects* | | |
| Cushing, *n* (%) | 47 (52) | 47 (44) |
| Striae, *n* (%) | 8 (9) | 8 (7) |
| Delta height SDS, median (IQR) | −0.18 (−0.32 to −0.01) | −0.17 (−0.38 to 0.01) |
| Delta behavior, VAS score, mm, median (IQR) | | |
| Unhappy | −2 (−21 to19) | −1 (19 to 21) |
| Trouble sleeping | 1 (−16 to 17) | 1 (15 to 17) |
| Hungry | 21 (1 to 38) | 19 (1 to 36) |
| Overactive | 18 (1 to 38) | 13 (−4 to 38) |
| Aggressive | 13 (1 to 49) | 8 (−1 to 39) |
| Severe infection, *n* (%) | 13 (14) | 14 (13) |
| Dyspepsia, *n* (%) | 3 (3) | 3 (3) |

Abbreviations: GR SNPS, glucocorticoid receptor single-nucleotide polymorphisms; IQR, interquartile range; SDS, standard deviation scores; VAS, visual analog scale; VLD-DST, very low dose dexamethasone suppression test.
Cumulative prednisolone dose was equal in both initial treatment regimes.

### Genotype distribution

Genotype distributions were in Hardy–Weinberg equilibrium. Figure 1 shows haplotype frequencies of the GR. The minor allele of the GR-9β polymorphism always coincided with the minor allele of the TthIII-1 polymorphism. Only the GR-9β + TthIII-1 (further referred to as the GR-9β haplotype) and *Bcl*I haplotypes were included in further analyses, as these showed allele frequencies of at least 15% (15.9 and 25.2%, respectively). As explained below, patients harboring either one or two copies of a particular haplotype were characterized as carriers, and those with zero copies as noncarriers.

### Glucocorticoid receptor gene polymorphisms in relation to clinical outcome

Carriers of the GR-9β haplotype had worse therapeutic outcome than noncarriers. At least one relapse occurred in 23 out of 25 carriers (92%) and in 56 out of 75 (75%) noncarriers, log rank test $P = 0.029$, empirical *P*-value (*P*-emp) = 0.036 (Figure 2a). Median time to the first relapse