

1 **Glucocorticoid pharmacogenetics in pediatric idiopathic nephrotic syndrome**

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31 Abstract

32 Idiopathic nephrotic syndrome (INS) represents the most common type of primary glomerular disease
33 in children: glucocorticoids (GCs) are the first line therapy, even if considerable inter-individual differences in
34 their efficacy and side effects have been reported. Immunosuppressive and anti-inflammatory effects of
35 these drugs are mainly due to the GC-mediated transcription regulation of pro- and anti-inflammatory genes.
36 This mechanism of action is the result of a complex multi-step pathway that involves the glucocorticoid
37 receptor and several other proteins, encoded by polymorphic genes. Aim of this review is to highlight the
38 current knowledge on genetic variants that could affect GC response, particularly focusing on children with
39 INS.

40

41 Keywords

42 Glucocorticoids; idiopathic nephrotic syndrome; polymorphisms; glucocorticoid receptor; glucocorticoid
43 receptor heterocomplex; inflammatory mediators; P-glycoprotein.

44

45 Idiopathic nephrotic syndrome (INS) is the most frequent primary glomerular disease in the pediatric
46 population, and affects 16 - 17 per 100.000 children. The onset of the disease occurs usually between the
47 ages of 2 and 8 years, with a peak of incidence between 3 and 5 years [1, 2]. The physiopathologic
48 mechanisms of INS have not been completely clarified yet; however, the disease is triggered by an increase
49 in glomerular permeability caused by an abnormal immunologic response, that results in an alteration of the
50 capillary structure and of the integrity of the glomerular membrane [1].

51 Glucocorticoids (GCs) are the mainstay of INS therapy. Response to GCs is highly correlated to
52 histological subtypes of the disease, and is poor in genetic forms that occur either as isolated kidney disease
53 or as syndromic disorders. Several gene mutations have been associated to these hereditary forms, in
54 particular variations in genes encoding for glomerular proteins such as nephrin (*NPHS1*), podocin (*NPHS2*),
55 phospholipase C epsilon-1 (*PLCE1*), Wilms Tumor gene (*WT1*), CD2-associated protein (*CD2AP*) and
56 others (for a review see [3]).

57 Also in non-genetic forms of INS, patients' response to GCs is the best indicator for outcome: indeed,
58 those who respond poorly to these drugs and do not achieve remission have an unfavourable prognosis and
59 often develop end-stage renal failure [4]. In minimal change nephrotic syndrome, the most common
60 histopathological pattern in children, accounting for 70-80% of cases [2], after an initial response to
61 prednisone, around 80% children relapse and some become steroid-dependent, while others never respond
62 to GC therapy and are therefore steroid resistant (10%). These patients often require intensified
63 immunosuppression with cyclophosphamide and/or cyclosporin A [1] [5].

64 This variable response to GCs is likely not attributable to the characteristics of the disease, and is
65 clinically difficult to predict. Significant advances have been made over the past years in understanding the
66 molecular basis of inter-patient variability: recent investigations have led to the hypothesis that genetic
67 factors influencing the patient pharmacokinetic or pharmacodynamic profiles may account for 20% to 95% of
68 variability in the efficacy and side effects of therapeutic agents [6]. Pharmacogenetics has therefore a
69 promising role in personalized medicine, hopefully allowing the identification, a priori, of treatment sensitive
70 and resistant patients and ensuring the right drug and right dose for each of them. In the context of INS, little
71 is known about the impact of genetic polymorphisms on steroid response. Nonetheless, identification of
72 predictive genetic biomarkers would be extremely beneficial, in particular for children with a steroid resistant
73 disease, preventing their exposure to ineffective drug courses.

74

75 This review describes the mechanisms of GC action and discusses the molecular and genetic basis of
76 GC resistance, with particular reference to non-genetic forms.

77

78 **MOLECULAR MECHANISM OF GC ACTION (Figure 1)**

79 GCs are anti-inflammatory and immunosuppressive drugs that exert their molecular action through
80 both genomic and non-genomic mechanisms. Depending on whether or not they modulate gene
81 transcription, GC induced effects could be delayed in onset but long-lasting or, vice versa, of more rapid
82 onset and shorter duration.

83

84 Genomic mechanisms

85 Exogenous and endogenous GCs are lipophilic substances that diffuse across plasma membranes,
86 thus interacting with a cytosolic receptor (the glucocorticoid receptor, GR), expressed in virtually all tissues.
87 This receptor is a member of the large nuclear receptor superfamily, which includes receptors for steroid
88 hormones and other hydrophobic molecules [7]; all these receptors are highly homologous to each other and
89 have a common modular domain organization with a transactivation domain at the N-terminal part (NTD), a
90 central zinc finger DNA-binding domain (DBD) and a ligand-specific binding domain (LBD) at the C-terminus.
91 In the cytoplasm, the ligand-free GR exists in a multimeric complex associated with various chaperones and
92 co-chaperones, such as the heat-shock proteins Hsp90, FKBP51, FKBP52, p23, Hsp70 and Hsp70/Hsp90
93 organizing protein (Hop) [8], that keep the receptor in the correct folding for hormone binding [9]. Upon
94 binding, the receptor undergoes conformational changes and exposes the DBD and the nuclear localization
95 signals, both hidden in the ligand-free conformation. The nuclear localization signals interact with
96 transporters located on nuclear membranes (the importins), thus mediating the GR translocation into the
97 nucleus. Once there, the DBD interacts, through its zinc finger motifs, with specific DNA sequences located
98 within regulatory regions of GC-responsive genes, the GC-responsive elements (GRE), [10] [11]. The GR
99 homodimerizes on GREs and recruits transcriptional co-activators and basal transcription machinery to the
100 transcription start site. These co-activators, that include CREB (cAMP response element-binding) binding
101 protein (CBP), steroid receptor co-activator-1 (SRC-1), GR-interacting protein (GRP-1) and the transcription
102 factors p300 and switching/sucrose non fermenting (SWI/SNF), induce histone acetylation and thus the
103 transactivation of GC-responsive genes (mediated by positive GREs). Through the induction of anti-
104 inflammatory genes, such as interleukin (*IL*)10, *annexin 1* and the inhibitor of nuclear factor (*I-κB*),

105 transactivation is responsible for some of the GCs anti-inflammatory effects [12, 13]; however,
106 transactivation enhances mainly the expression of genes involved in metabolic processes [14, 15], and is
107 therefore responsible for the majority of side effects related to GC administration [16, 17]. In contrast,
108 negative GREs [18] mediate downregulation of transcription of responsive genes and transrepression is
109 responsible for the majority of the beneficial anti-inflammatory effects of GCs [16, 19-21]. Furthermore, GRE-
110 independent mechanisms of transrepression also exist: the GR physically interacts and inhibits AP-1 [22]
111 and nuclear factor (NF)- κ B [23], two important transcription factors involved in the pro-inflammatory
112 mechanism.

113

114 Non genomic mechanisms

115 Non genomic mechanisms have been also described and are responsible for the effects induced by
116 GCs characterized by rapid onset and short duration. The mechanisms are still not completely clear, but
117 likely involve non-classical membrane-bound GC receptors. In addition, at higher concentrations, GCs
118 probably induce lipid peroxidation, with consequent alteration of the characteristics of plasma membranes
119 and alteration in ion transport [24].

120

121 **MOLECULAR MECHANISM OF GC RESISTANCE**

122 The precise molecular mechanism conferring dependence or resistance to GCs in INS and in other
123 diseases is still unclear; likely, the mechanism is not unique and probably occurs after impairments at
124 different levels such as: 1) the GR receptor heterocomplex and proteins involved in nuclear translocation; 2)
125 the pro- and anti-inflammatory mediators in the downstream signalling pathway of the GC-GR complex; 3)
126 the P-glycoprotein (P-gp), an efflux transporter of GCs, and the drug-metabolizing enzyme CYP3A5.

127

128 **1. The GR heterocomplex and proteins involved in nuclear translocation**

129 *The GR*

130 The *NR3C1* gene, encoding for the human GR, is located on chromosome 5q31.3 and includes nine
131 exons [25]. Several polymorphic sites have been described in this gene and have been supposed to affect,
132 at least partially, the inter-patient variability in GCs response because they might alter the formation and the
133 dynamic of the GC–GR complex and hence the downstream gene expression regulation [26]. However, only
134 few variants have been associated with differences in metabolic parameters, body composition and altered

135 endogenous cortisol levels and are functionally relevant [26-37]. Single nucleotide polymorphisms (SNPs)
136 such as *TthIII* (rs10052957), ER22/23EK (rs6189/rs6190) and GR-9 β (rs6198), have been related to a
137 reduced sensitivity to endogenous and exogenous GCs, while other *NR3C1* SNPs such as N363S (rs6195)
138 and *Bcl* (rs41423247) have been related to an increased sensitivity [26, 37]. *TthIII* is a C>T change in the
139 *NR3C1* promoter region, located 3807 bp upstream of the GR start site [9]; the ER22/23EK polymorphisms
140 involve two nucleotides changes (GAGAGG to GAAAAG) in codon 22 and 23 of *NR3C1* exon 2, which
141 change the amino acid sequence of the NTD domain from glutamic acid-arginine (E-R) to glutamic acid-
142 lysine (E-K) [38]; the GR-9 β polymorphism is located in the 3'-untranslated region of exon 9 β , where an
143 ATTTA sequence is changed into GTTTA [39]. The N363S polymorphism consists of an AAT>AGT
144 nucleotide change at position 1220 in exon 2, resulting in an asparagine to serine change in codon 363 [40],
145 the *Bcl* polymorphism was initially described as a polymorphic restriction site inside intron 2, and the
146 nucleotide alteration was subsequently identified as a C>G substitution, 646 nucleotides downstream from
147 exon 2 [41].

148 So far, only few studies have evaluated the role of the *NR3C1* polymorphisms on the response to
149 exogenous GCs in patients affected by INS. The distribution of *Bcl* and of two other SNPs, rs33389 and
150 rs33388, (respectively a C>T and A>T substitution, 76889 and 80093 nucleotides downstream from exon 2)
151 also located in intron B of the GR receptor gene, as well as the three-marker haplotype, has been studied in
152 136 healthy children and 118 INS pediatric patients who initially responded to oral GC therapy. The GTA
153 haplotype was associated with a higher steroid sensitivity, determined by time to proteinuria resolution, and
154 was more prevalent in early (response \leq 7 days) than late (response > 7 days) prednisone responders (27.7
155 vs 14.5%, hap-score = -2.22, p = 0.05) [42]. The *Bcl* polymorphism has been also analysed by Cho and co-
156 workers [43] in 190 Korean children with INS and 100 controls, but no correlation with the development of
157 INS, onset age, initial steroid responsiveness, renal pathologic findings and the progression of renal disease
158 was found. The authors have also examined two other SNPs, namely ER22/23EK and N363S, but no variant
159 allele was found in any of the patients or control subjects. Recently, Teeninga et al. [44] have evaluated GR-
160 9 β , *TthIII* and *Bcl* polymorphisms in a well-defined cohort of 113 children with INS, showing that carriers of
161 GR-9 β +*TthIII* mutated haplotype had a significantly higher incidence of steroid dependence compared with
162 non-carriers (52% vs 25%, OR = 3.04 95% CI 1.37–6.74, log rank test p = 0.003).

163 Several GR protein isoforms are generated through an alternative splicing: the most abundant and
164 functionally active isoform is GR α , whereas GR β is the inactive protein, unable to bind the ligand that exerts

165 a dominant negative effect on GR α . The GR-9 β polymorphism has been associated with increased
166 expression of the mature GR- β protein and implicated in steroid resistance in several diseases [45-49]. In
167 patients with INS, an increased expression of GR β has been demonstrated in peripheral blood mononuclear
168 cells (PBMCs) of steroid resistant patients [50], while the expression of the functional isoform GR α was
169 correlated with a positive steroid response (steroid responders vs partial- and non-responders $p < 0.01$) [51].

170 In 2006, Ye et al. [52] sequenced candidate exons of *NR3C1* gene and examined all the genetic
171 variations in 138 Chinese children with sporadic steroid resistant and sensitive INS, founding no significant
172 association between the SNPs analysed in the study and steroid response; however the analysis excluded
173 the above mentioned polymorphisms that are located in *NR3C1* introns and regulatory regions.

174

175 *The GR heterocomplex*

176 Beside the proper functioning of the receptor itself, also the activity of all other components in the GR
177 heterocomplex is essential for an adequate response to GCs. Altered levels of heterocomplex proteins, such
178 as Hsp90, Hsp70, FKBP51, FKBP52, p23 and Hop, may contribute to altered GC cellular sensitivity [53] [54].
179 In INS, Ouyang et al. [55] have shown that the expression level of Hsp90 mRNA was significantly higher in
180 adult patients than in healthy controls (1.09 ± 0.17 vs 0.98 ± 0.14 , $p < 0.05$), and both the expression and
181 nuclear distribution of Hsp90 were increased in PBMCs obtained from GC-resistant patients in comparison to
182 GC-sensitive ones (1.28 ± 0.25 vs 1.13 ± 0.21 ; $p < 0.05$). The same authors have subsequently explored the
183 interaction between Hsp90 and the GR in the nucleus as well as the DNA binding activity of the GR, showing
184 that the nuclear enrichment rather than total cellular expression of Hsp90 might contribute to GC resistance
185 and that the DNA binding activity of the GR was significantly ($p < 0.05$) decreased in GC resistant patients,
186 hindering transactivation [56].

187 Clinical studies on the association between variants in genes coding for GR heterocomplex proteins
188 and the GC response have been already carried out in several GC-treated diseases. In inflammatory bowel
189 disease Maltese et al. [57] analyzed the role of *FKBP5* genetic variants (rs3800373, rs1360780 and
190 rs4713916) and evidenced that the variant rs4713916 polymorphism was significantly associated with
191 resistance to GC treatment in Crohn's disease (responders = 17% vs resistants = 35%; $p = 0.0043$).
192 Moreover, in a cohort of asthmatic patients, Hawkins et al. [58] analyzed the role of *FKBP5* genetic variants
193 in response to GCs, however the studied polymorphisms (rs3800373, rs9394309, rs938525, rs9470080,
194 rs9368878 and rs3798346) were not correlated with response to these drugs. In the same study, genetic

195 variations in the *STIP1* gene (rs4980524, rs6591838, rs2236647, rs2236648), which codes for Hop, have
196 been investigated and shown to have a role in identifying asthmatic subjects who were more responsive to
197 GC therapy. An association with improved lung function, evaluated as baseline FEV1 (rs4980524, p = 0.009;
198 rs6591838, p = 0.0045; rs2236647, p = 0.002; and rs2236648; p = 0.013) was found [58]. To date, no data
199 on these polymorphisms and therapeutic outcome in INS are available. Pharmacogenetic studies are
200 therefore required in order to understand the importance of these genetic variants in identifying resistant
201 patients in this condition.

202

203 *Nuclear transport factors*

204 Upon binding with the receptor, the GR-GC nuclear translocation is essential to exert the GC
205 pharmacological function, and this step is mediated by several nuclear receptors known as importins. [59]
206 [60]. Importin 13 (IPO13) has been functionally characterized as a primary regulator of GC-bound GR across
207 the nuclear membrane [10]. Altered levels of this protein might affect the therapeutic responsiveness to GCs
208 and it has been demonstrated that *IPO13* silencing prevents GC transport across the cytoplasmic-nuclear
209 membrane in airway epithelium and abrogates GC-induced anti-inflammatory responses [61]. SNPs in the
210 *IPO13* family have been associated with neonatal respiratory outcomes after maternal antenatal
211 corticosteroid treatment (SNP impact on fetal bronchopulmonary dysplasia: rs4448553; OR 0.01; 95% CI
212 0.00-0.92, p = 0.04; SNP impact on surfactant maternal therapy: rs2428953 OR, 13.8; 95% CI 1.80-105.5,
213 p= 0.01 and rs2486014 OR 35.5; 95% CI 1.71-736.6, p = 0.02) [62]. Polymorphisms of *IPO13* (rs6671164,
214 rs4448553, rs1990150, rs2240447, rs2486014, rs2301993, rs2301992, rs1636879, rs7412307 and
215 rs2428953) have been investigated in children with mild to moderate asthma in relation with clinical response
216 to GCs evidencing that *IPO13* variants could increase the nuclear bioavailability of endogenous GCs
217 (subjects harboring minor alleles demonstrate an average 1.51–2.17 fold increase in mean PC₂₀ at 8-months
218 post-randomization that persisted over four years of observation: p = 0.01–0.005) [63]. To date, no study on
219 *IPO13* genetic variants are available in INS patients, therefore investigation in this population is required.

220

221 **2. The pro- and anti-inflammatory mediators in the downstream signaling pathway of the GC–GR** 222 **complex**

223 INS was proposed as a T cell dysfunction disorder [64], although mechanisms by which T cells affect
224 the course of the disease are still unclear. Cytokines are released from activated T cells and play a crucial

225 role in the pathogenesis of INS [65] [66]; imbalances in T cells phenotypes, response and cytokines have
226 been found between steroid sensitive and resistant INS patients [67] as well as between those who relapse
227 and those in remission [68] [64].

228 Endogenous GCs are involved in the balance of pro- and anti-inflammatory mediators: a complex
229 circular interplay between GCs and cytokines takes place, with GCs downregulating pro-inflammatory
230 cytokines and cytokines limiting GC action [69] [70-72].

231 Basal cytokine expression levels are fine-tuned by genetic profile. Polymorphisms in the cytokine
232 genes involved in the pathogenesis of INS (among which *IL1*, *IL12*, tumor necrosis factor (*TNFA*),
233 macrophage migration inhibitory factor (*MIF*), *IL4*, *IL6* and *IL10*) and in glucocorticoid-induced transcript 1
234 gene (*GLCCI1*) might in part be responsible of inter-individual variations in therapy.

235

236 *Pro-inflammatory mediators*

237 **IL-1:** IL-1 family is a group of 11 cytokines among which IL-1 α and IL-1 β are the most studied. In
238 glomeruli affected by several forms of INS, podocytes are capable of producing IL-1 α/β [73]; however, the
239 role of IL-1 in the immunopathogenesis of INS is still controversial. Saxena et al. found that, in supernatants
240 of phytohaemagglutinin activated lymphocyte cultures obtained from patients with minimal change nephrotic
241 syndrome, IL-1 levels were increased when compared to controls [74], while other studies did not confirm
242 such finding. Chen and co-workers showed an overexpression of IL-1 at the protein and mRNA level in
243 glomerular mesangial cells of patients affected by IgM mesangial nephropathy but not in those with minimal
244 change nephrotic syndrome [75], and Suranyi et al. could not find differences between INS patients and
245 controls in IL-1 β levels measured in plasma, urine and culture supernatant of mitogen-stimulated PBMCs
246 [76].

247 Several polymorphisms in *IL1* genes have been described [77] and associated with altered levels of
248 the cytokine level [78]: T-31C (rs1143627) SNP results in the loss of the first T in TATA box and has been
249 observed to cause a paradoxical increase in IL-1 β in the presence of steroids in PBMCs under acute
250 inflammation [79]. The C-511T SNP (rs16944) has been correlated to loss of the binding site for the
251 transcription factor AP-2. Carriers of the haplotype composed of IL-1 β -31C allele and -511T allele have
252 showed a 2-3 fold increase in LPS-induced IL-1 β secretion measured by an ex-vivo blood stimulation assay,
253 the association was observed in two independent population ($p = 0.0084$ and $p = 0.0017$) [80, 81]; these

254 SNPs might therefore be of relevance in the modulation of GC response. So far, no data are available for
255 INS and studies that investigate this association should be carried out.

256

257 **IL-12:** IL-12 has also been implicated in the pathogenesis of INS; this cytokine is produced by antigen
258 presenting cells and regulates the growth and development of natural killer (NK) and T cells; in addition, it is
259 the major inducer of interferon (IFN)- γ [82].

260 IL-12 serum levels have been investigated in different cohorts of patients: Lin and Chien [83] studied
261 20 INS patients and found a significant increase of the cytokine in relapsed patients as compared to patients
262 in remission and to normal controls. The amount of IL-12 was also increased during the active phase of the
263 disease as compared to the remission and was reported to upregulate the production of vascular
264 permeability factor, a clinical index of INS [84, 85]. On the contrary, Stefanovic et al. did not find difference in
265 terms of IL-12 production between concanavalin A-stimulated PBMCs of 20 children with steroid sensitive
266 INS and 17 healthy control subjects [86].

267 Genetic variations in *IL12* gene have been investigated: a complex bi-allelic polymorphism in the
268 promoter region of the gene, coding for the p40 subunit (IL12B) has been described (IL-12Bpro,
269 CTCTAA/GC polymorphisms; rs17860508). IL-12Bpro allele 1 has been related to a reduced IL-12 secretion
270 in dendritic cells [8, 87]. Surprisingly, this allele had a high frequency in 45 steroid dependent INS children
271 (46.7%) compared to 34 non dependent (17.6 %; $p = 0.016$) [8].

272

273 **TNF:** TNF is a potent pro-inflammatory protein released by monocytes upon stimulation, being almost
274 undetectable in resting conditions [88]. The *TNFA* gene is located on chromosome 6p21.3, in the class III
275 region of the major histocompatibility complex within the human leukocyte antigen [89, 90], which contains
276 many genes involved in inflammatory and immune responses [91]. An increase in *TNFA* gene expression,
277 higher serum TNF levels and TNF production by monocytes has been demonstrated in INS patients with
278 active disease, in comparison with patients in remission and controls [92]. TNF was the only cytokine found
279 to be increased in plasma and urine in INS patients affected by segmental glomerulosclerosis and
280 membranous nephropathy, but not in those with minimal change nephropathy [76].

281 Among *TNFA* polymorphisms, the G-308A (rs1800629) is one of the best documented [93]. This SNP
282 lies in a binding site for the transcription factor AP-1 and the A allele has been shown to have higher
283 transcriptional activity than the G allele, increasing TNF production *in vitro* [94]. Conflicting results have been

284 reported for this polymorphism in patients with INS. A study by Kim and colleagues, on 152 patients with
285 childhood INS and 292 healthy adult controls, investigated the association between cytokine polymorphisms,
286 among which *TNFA* G-308A, and disease susceptibility, and did not find significant differences in allele
287 frequencies between the two populations [95]. This study is in contrast with other results that found a
288 significant association, both at genotypic and allelic level, with susceptibility and with steroid resistance.
289 Indeed, on comparing 115 GC sensitive and 35 GC resistant patients, the AA genotype was suggested as a
290 causative factor of non responsiveness to steroid therapy among INS children (responsive vs non-
291 responsive patients: at genotypic level OR = 14.71, 95% CI = 1.59-136.46, p = 0.0121; and at allelic level
292 OR = 2.251, 95% CI = 1.09-4.66, p = 0.0433) [96, 97].

293

294 **MIF:** MIF is also a pro-inflammatory cytokine with a pathogenic role in kidney diseases [98]. MIF is
295 produced by several cell types, particularly T cells but also monocytes, macrophages, glomerular epithelial
296 cells, tubular epithelial cells and vascular endothelial cells. Due to its regulatory properties on innate and
297 adaptive immune responses, MIF is considered a critical mediator in various immune and inflammatory
298 diseases [99-102]: its expression has been found to be increased in all forms of glomerulonephritis although
299 not in minimal change nephrotic syndrome [98].

300 MIF has the ability to override the inhibitory effects of GCs on the immune system: when present at
301 low levels, GCs up-regulate MIF, while at higher GC concentrations, a counter-regulatory mechanism is
302 observed and GCs down-regulate this cytokine expression [103, 104]. The *MIF* gene is located on
303 chromosome 22q11, and recently a G-173C (rs755622) polymorphism, that involves a G to C substitution at
304 base pair 173 of the 50-flanking region, was found to be strongly associated with higher MIF expression *in*
305 *vitro* [101]. Berdeli et al. [105] and Vivarelli et al. [106] have investigated this polymorphism in Turkish and
306 Italian children with INS (214 and 257 respectively) and found that the frequency of the C allele was higher in
307 patients than in controls (19 vs 8%, OR=2.5, 95 CI% 1.4–4.2, p = 0.0007 [105] and 32 vs 22% OR=1.67,
308 95% CI 1.16–2.41; p=0.006 [106]); in addition, the polymorphism was significantly more frequent in steroid
309 resistant patients than in sensitive ones (33 vs 12% OR=3.6, 95 CI% 2.2–6.0, p < 0.0001 [105] and 44 vs
310 23% OR 2.61, 95% CI 1.52–4.47; p=0.0005 [106]). Interestingly Choi et al. [107], investigating the same
311 SNP in 170 Korean children with INS could not find any association between the G-173C polymorphism and
312 clinical parameters, renal histological findings and steroid responsiveness.

313 Moreover, in a recent study, Swierczewska et al. [108] investigated the role of seven other
314 polymorphic variants of the *MIF* gene: two polymorphisms, rs2070767 (C>T) and rs2000466 (T>G), were
315 found to have a significantly different distribution between 30 resistant and 41 sensitive INS patients
316 (rs2070767, CT vs CC, OR=3.00, 95 CI% 1.043-8.627, p=0.047; rs2000466, TG+GG vs TT, OR=0.321, 95
317 CI% 0.119-0.869, p=0.028); however, when linkage disequilibrium analysis was performed, the significance
318 was lost.

319 Finally, a recent meta-analysis of Tong and colleagues [109], considering all the articles cited before,
320 confirmed that *MIF* G-173C polymorphism may increase the risk of renal disease and may be associated
321 with GCs resistance in INS, especially in children. The pooled results, considering eight case-control studies
322 and 2755 participants, indicated a significant association between *MIF* -173G/C polymorphism and renal
323 disease risk (CC+CG vs GG, OR = 1.77, P < 0.01; C vs G, OR = 3.94, P < 0.01).

324

325 *Anti-inflammatory mediators*

326 **IL-4:** IL-4 is a potent anti-inflammatory [110] and a key cytokine involved in the development of allergic
327 diseases, being required, together with other cytokines, for the class switching of B cells to immunoglobulin
328 E (IgE) production [111]. INS is frequently associated with allergic symptoms and elevated serum IgE levels
329 [112]. Increased serum IL-4 levels have been observed in patients with INS [113] and in particular in steroid
330 sensitive patients in active stage compared with those in remission (p=0.033) and with healthy controls,
331 (p=0.011) [68]; similar results were obtained by Prizna et al. in INS patients with active stage in comparison
332 with patients in remission on steroids (p < 0.0001), in remission off steroids (p < 0.0001) and controls (p <
333 0.0001) [114].

334 Genetic variants in *IL4* may be associated with predisposition to INS, and to the clinical course of the
335 disease [115-117]. A C>T exchange at position 590 upstream from the open reading frame of the *IL4* gene
336 (rs2243250) has been shown to be associated with elevated levels of IgE [118]. Tripathi et al. [97]
337 demonstrated that this polymorphism influences the prognosis of the disease: indeed, the TT genotype was
338 more frequent in 35 children with steroid resistant INS as compared to 115 steroid sensitive (OR = 7.29, 95%
339 CI = 1.26-41.69, p = 0.0386). This observation was subsequently confirmed by Jafar et al. in a cohort of 150
340 INS children (OR = 6.46, 95 CI% 1.11–37.66, p = 0.020) [96].

341 IL-4 signaling is mediated by the interaction of the cytokine with its receptor, mainly expressed in
342 hematopoietic cells. The distribution of the IL-4 receptor α chain genetic polymorphism Ile50Val (rs1805010)

343 was studied in 85 Japanese INS patients grouped according to the number of relapses: the mutated
344 genotype was significantly less frequent in patients who experienced four or more relapses (3.3%) compared
345 to those who experienced three or less recurrences (29.8%, $p = 0.007$) [119]. However, these data were not
346 confirmed by Tenbrock et al. [120] who could not find an association between patient genotypes and INS
347 clinical courses (measured as frequent relapses (29 children) and steroid dependence (35) or resistance
348 (11)).

349

350 **IL-6:** IL-6, a multifunctional cytokine that plays a central role in host defenses [121], and has both
351 pro- and anti-inflammatory effects. In INS, plasma levels of this cytokine were associated to disease
352 susceptibility, being increased in patients compared to controls [122], and to treatment responsiveness,
353 being enhanced in steroid resistant patients compared to steroid sensitive and controls ($p < 0.05$) [123].

354 The *IL-6* gene, located on chromosome 7p21-24, presents different polymorphisms. Among these, the
355 common G>C SNP at position -174 in the promoter region, influences the transcriptional regulation and the
356 cytokine plasma levels in different renal diseases [124, 125]. Tripathi et al. [97] found that the GG genotype
357 was more frequent in 35 INS steroid resistant children (11.4%), as compared with 115 steroid sensitive
358 patients (0.9%; OR = 14.71, 95% CI = 1.59-136.46, $p = 0.0121$). These results have been confirmed by Jafar
359 et al. [96] (OR = 31.40, 95% CI = 3.62–272.3, $p < 0.001$) suggesting that this polymorphism could be a
360 causative factor for non-responsiveness toward steroid therapy among INS children.

361

362 **IL-10:** IL-10, known as human cytokine synthesis inhibitory factor, is produced primarily by monocytes
363 and to a lesser extent by lymphocytes. IL-10 has pleiotropic effects in immunoregulation and inflammation
364 [126] [127]; it inhibits the production of inflammatory mediators, and can be considered as a natural
365 immunosuppressant of TNF [128].

366 GCs upregulate the expression of IL-10 [69], that in turn acts synergistically with GCs, as
367 demonstrated in whole-blood cell cultures where the presence of IL-10 improved the ability of
368 dexamethasone to reduce IL-6 secretion. In addition, the cytokine increased the concentration of
369 dexamethasone-binding sites in these cells, with no effect on the binding affinity [126].

370 IL-10 expression was significantly reduced in T regulatory cells from adult INS patients (10.3 ± 3.4
371 pg/ml) compared to healthy donors (19.3 ± 5.9 pg/ml; $p < 0.01$) [129]; similar results were obtain by Araya

372 and colleagues; $p < 0.0191$) [130], while no significant difference was found between IL-10 serum levels of
373 INS pediatric patients in nephrotic phase (heavy proteinuria) and in remission [111].

374 The human *IL10* gene is located on chromosome 1q31–q32. Previous studies have demonstrated that
375 an A>G polymorphism at nucleotide position –1082 in the promoter region (rs1800896) influences the IL-10
376 transcriptional levels. The mutated genotype has been associated with significantly higher cytokine plasma
377 levels in acute lymphoblastic leukemia patients [131], as well as with a positive prednisone response in
378 childhood acute lymphoblastic leukemia [33, 131] and in patients with rheumatoid arthritis [132].

379 To authors' knowledge, association of *IL10* polymorphisms and the response to steroid therapy in INS
380 has never been investigated; in a pharmacogenetic study on rs1800896, the GA/GG genotypes have been
381 associated, in 191 patients, with the progression of the disease in both IgA nephropathy and focal segmental
382 glomerulosclerosis (the GA/AA genotypes was over-represented in fast progressors: OR = 1.25, 95% CI
383 1.07–1.47, $p = 0.012$) [133].

384

385 **GLCCI1**: *GLCCI1* was initially identified as a transcript rapidly up-regulated in response to GC
386 treatment in cells derived from a thymoma [134]. In the kidney, it is expressed specifically in mesangial cells
387 and podocytes and knockdown of the transcript impairs the glomerular filtration barrier in developing
388 zebrafish [135]. Recently in a genome-wide association study, which examined the response to inhaled GCs
389 in 1041 asthmatic patients, two SNPs (rs37972 and rs37973) in complete linkage disequilibrium in the
390 promoter region of *GLCCI1* have been associated with a poorer response to steroid treatment (OR = 1.52,
391 95% CI = 1.13 - 2.03) [136].

392 Cheong and colleagues [137] genotyped 211 pediatric patients with INS and 102 controls for the
393 rs37972 and rs37973, and did not find any statistically significant associations between the SNPs analyzed
394 and either the development of INS, or initial response to steroid therapy.

395

396 **3. P-glycoprotein (P-gp) and drug metabolizing enzyme CYP3A5**

397 *P-glycoprotein*

398 P-gp is a 170-kDa ATP dependent membrane transporter, an efflux pump responsible for resistance to
399 a number of structurally and functionally unrelated drugs, including natural and synthetic GCs [138], that are
400 actively exported from cells against the concentration gradient [139]. Several studies have been conducted
401 to evaluate the association of P-gp expression with the responsiveness to GCs in many diseases among

402 which INS: Wasilewska et al. [140] found that P-gp expression in CD3 positive lymphocytes was significantly
403 higher in patients with INS than in controls ($p = 0.0004$). A significant difference was also observed between
404 controls (1.24 ± 0.58) and both steroid dependent (7.00 ± 3.09 , $p = 0.0001$), and the frequent relapsing group
405 (5.56 ± 4.07 , $p = 0.0002$); while the difference with the non frequent relapsing group was smaller ($p < 0.05$).
406 Moreover a significant difference was observed between non frequent relapsing (3.02 ± 3.46) and both
407 steroid dependent ($p < 0.001$) and frequent relapsing group ($p < 0.001$) [141]. P-gp mRNA expression levels
408 in PBMCs were found to be variable in patients with INS prior to remission, but decreased after complete
409 remission ($p < 0.003$) [142]. In another study by Stachowski et al. [143], mRNA expression in peripheral
410 lymphocytes of patients with steroid, cyclophosphamide or cyclosporine resistant INS was higher than in
411 lymphocytes from patients who were sensitive to these drugs ($p < 0.001$). Moreover, in a recent work,
412 Prasad et al. [68] found that steroid therapy in INS decreased P-gp expression in peripheral blood
413 lymphocytes (absolute P-gp expression at baseline 66.59 ± 21.13 vs remission 35.84 ± 22.26 , $p < 0.05$).

414 P-gp is encoded by the ATP-Binding Cassette, sub-family B (*ABCB1*; multi drug resistant protein 1
415 *MDR1*) gene, located on human chromosome 7q21.12 [144], and several studies have demonstrated that
416 genetic polymorphisms in this gene lead to functional alterations and are associated with altered drug
417 disposition [145, 146]. A synonymous SNP in exon 26 (C3435T, rs1045642) was the first variation to be
418 associated with altered protein expression [145]. SNPs at exons 12 (C1236T, rs1128503), 21 (G2677T/A,
419 rs2032582) and 1b (T-129C, rs3213619) may also be associated with altered transport function or
420 expression [147].

421 In 108 pediatric INS patients, Wasilewska et al. [148] have studied the association between C1236T,
422 G2677T/A and C3435T polymorphisms and the clinical course and treatment response. All individual
423 polymorphisms were strongly associated with time to response to initial prednisone therapy (OR = 6.79, 95%
424 CI: 1.96-23.54, $p < 0.001$ for 1236 T/T, OR = 13.7, 95% CI: 2.78–67, $p < 0.001$ for 2677 T/T and OR = 9.92,
425 95% CI: 3.01–32.71, $p < 0.001$ for 3435 T/T), and the frequencies of the mutated allele were higher in late
426 responders (53%, 52%, 66% for the C1236T, G2677T/A and C3435T polymorphisms respectively) than in
427 early responders (24%, 19%, 32%). The TTT haplotype was also significantly associated with late steroid
428 response compared to early response (49% vs. 19%, $p = 0.0003$).

429 More recently, Choi et al. [107] have investigated the same polymorphisms (C1236T, G2677T/A and
430 C3435T) in 170 Korean children with INS, finding that the frequencies of the TGC haplotype was significantly
431 lower in the initial steroid responders (115 children) than in non-responders (35) (15.8 vs 29.0%; OR 0.46,

432 95% CI 0.27–0.78, $p=0.004$). Jafar et al. [149], in 216 patients with INS and 216 controls, found that the
433 homozygous mutations of G2677T/A SNP was associated with steroid resistance (18% steroid resistant vs
434 6% steroid responsive OR = 3.39, 95% CI 1.29–8.93, $p = 0.011$) and that the combination of mutated
435 genotype of SNP G2677T/A and C3435T synergistically increased the risk of developing steroid resistance
436 in patients with INS (5% in steroid resistant patients, 2% in steroid responsive and 1% in controls, $p = 0.038$).

437 Chiou et al. [150] also investigated in 74 children with INS the same polymorphisms. They could find
438 only a significant association of C1236T polymorphism with steroid resistance: the frequency of the T allele
439 was significantly higher in steroid resistant patients than in sensitive ones (81 vs. 62%; OR = 2.65, 95 % CI
440 1.01-6.94; $p = 0.042$).

441 In a recent study Youssef et al. [151] evidenced that the mutated and heterozygous G2677T/A
442 variants were significantly more frequent in 46 non-responders INS patients (28%) than in 92 responders
443 (20%; OR = 2.9, 95% CI 0.95–9.21, $p = 0.016$). Finally Cizmarikova et al. [152] also found in 46 INS patients
444 a significantly increased chance of therapeutic response in children carrying the 3435CT genotype (OR =
445 5.13, 95% CI 1.18-22.25, $p = 0.022$).

446 As shown in Table 1, P-gp has been largely studied in INS patients, and the results seem to be the
447 most coherent among the polymorphisms studied in this disease.

448

449 *CYP3A5*

450 The human cytochrome P450 (CYP) family comprises a number of CYP isoforms that have important
451 functions in the reductive and oxidative metabolism of many endogenous and exogenous compounds,
452 among which steroids. CYP3A5*3 is an A to G transition (A6986G) within intron 3 of *CYP3A5* gene that
453 creates an alternative splice site in the pre-mRNA, producing an aberrant mRNA with a premature stop
454 codon. CYP3A5*3 homozygotes (GG genotype) lack CYP3A5 expression, while individuals with at least one
455 CYP3A5*1 wild-type allele (AA and AG genotypes) express the protein [153]. In a recent study of Chiou and
456 colleagues, authors investigated polymorphic expression of CYP3A5 in 74 children with INS: the frequency
457 of the G allele (A6986G SNP) was relatively higher in steroid resistant subjects than in steroid sensitive ones
458 showing a trend of association, that however did not reach statistical significance (OR 2.63, 95 % CI 0.94–
459 7.37; $p=0.059$) [150].

460

461 Genetic polymorphisms of *CYP3A5* and *ABCB1* could have a role on the pharmacokinetics of
462 prednisolone; in particular, intestinal *CYP3A5* and P-glycoprotein are important in the absorption, systemic
463 drug distribution and cellular accumulation of glucocorticoids. However, a study of Miura et al. [154] found
464 only a small effect of *CYP3A5* and *ABCB1* genetic polymorphism on prednisolone pharmacokinetics.
465 Intracellular accumulation of GCs within lymphocytes, influenced by the expression of P-gp on these cells, is
466 probably more important and could influence steroid response in INS.

467

468 **CONCLUSION**

469 GCs are used in the treatment of active INS to induce remission of proteinuria, but inter-individual
470 differences in their efficacy and side effects have been reported. A main goal for clinicians is therefore to
471 improve the efficacy and safety of these agents and, when possible, to reduce steroid exposure. This is
472 particularly important in patients that do not respond and will suffer considerable steroid side effects without
473 any clinical gain, or in patients that will be dependent to steroid treatment and will not be able to withdraw the
474 drug, in whom switching to other therapy as soon as possible could be very important. Molecular
475 mechanisms involved in variability in GC response are still not completely known, but advance in
476 pharmacogenomics could contribute to the optimization and personalization of therapy.

477 This review is about the current literature on the molecular mechanisms of GC anti-inflammatory
478 action and the role of genetic polymorphisms in variable GC response in patients with INS. Results of
479 reported papers are not conclusive and often in contradiction, and at present none of the potential
480 pharmacogenetic markers is strong enough to be used in clinical practice.

481

482 **FUTURE PERSPECTIVES**

483 In the future, beside candidate gene approach it would be necessary to perform sequencing of all the
484 genes involved in the GC mechanism of action, to obtain new comprehensive information. Recently, genetics
485 have focused the attention on copy number variation (CNV) and DNA methylation analyses. CNVs are
486 genomic alterations that result in the cell having an abnormal number of copies of one or more sections of
487 the DNA. Some CNVs have already been associated with susceptibility to diseases or response to drug
488 therapy but, until now, no data are available for GCs in relation to clinical response. In addition, DNA
489 methylation of gene promoters has been associated with transcriptional inactivation: changes in DNA
490 methylation can lead to differences in gene expression levels and thereby influence drug response. All these

491 approaches need to be performed in larger and well-characterized patient cohorts, uniformly treated and
492 systematically evaluated, and subsequently validated in other independent cohorts.

493 In conclusion, these new strategies for the identification of pharmacogenetic determinants associated
494 with GC response in paediatric INS patients, and the consequent personalization of therapy based on this
495 information, will result in higher quality and less toxic treatment of children, avoiding inadequate regimens or
496 time wasting and reducing overall health costs.

497

498 Executive Summary

- 499 • INS is the most frequent primary glomerular disease in the pediatric population and GCs are the first
500 line therapy in these patients. However there is a considerable inter-individual variability in response
501 to GCs that is clinically difficult to predict.
- 502 • Genetic factors could influence GC response, therefore pharmacogenetics has a promising role in
503 personalized medicine even if, to date, not conclusive results have been reported for steroid clinical
504 response.
- 505 • Several polymorphisms in genes involved in GC molecular mechanism (GR heterocomplex, pro- and
506 anti-inflammatory mediators and P-gp) could affect GC response in INS patients.

507 GR heterocomplex

- 508 • The *NR3C1 Bcl*, rs33389 and rs33388 SNPs have been associated with a higher steroid sensitivity
509 while GR-9 β and *TthIII* haplotype was associated with steroid dependence.
- 510 • The expression level of Hsp90 mRNA was increased in PBMCs obtained from GC-resistant patients
511 in comparison to GC-sensitive ones. On the contrary, to date, no data on *Hsp90*, *FKBP51*, *FKBP52*,
512 *p23*, *Hop* and *IPO13* gene polymorphisms and therapeutic outcome in INS are available;
513 pharmacogenetic studies are therefore still required.

514 Pro- and anti-inflammatory mediators involved in INS pathogenesis

- 515 • A complex bi-allelic polymorphism in the promoter region of the gene coding for the p40 subunit of
516 *IL-12* gene has a higher frequency in steroid dependents compared to steroid responders.
- 517 • The *TNF- α* G-308A polymorphism has also been investigated and the AA genotype has been
518 suggested to be a causative factor of non responsiveness to GC therapy.
- 519 • *MIF* G-173C polymorphism may increase the risk of renal disease and may be associated with GCs
520 resistance risk especially in children.
- 521 • The *IL-4* C590T mutated genotype has been associated with steroid resistance in children with INS.
- 522 • The wild type genotype of G-174C polymorphism in *IL-6* gene has been suggested to be a causative
523 factor for GC non-responsiveness.

524 P-glycoprotein (P-gp)

- 525 • Variant genotypes in *ABCB1* gene (C3435T,G2677T/A, C1236T) alone and in haplotype have been
526 correlated with steroid resistance.

527

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918 Legend to the figure
919 Figure 1: Molecular mechanisms of action of glucocorticoids.
920
921 Table 1
922 Summary of studies reporting genetic analysis of NR3C1 in INS patients.
923
924 Table 2
925 Summary of studies reporting genetic analysis of pro- and anti-inflammatory mediators in the
926 downstream signaling pathway of the GC-GR complex in INS patients.
927
928 Table 3
929 Summary of studies reporting genetic analysis on the role of P-gp in INS patients.

First author	Year	Ethnicity	Case/Control	Age (mean)	Results
Results for genetic analysis of NR3C1 in INS patients					
Zalewski G et al. [42]	2008	Caucasian (Poland)	118/136	5.1/NA	<i>BclI</i> (G>C), rs33389 (C>T) and rs33388 (A>T) GTA aploptype was associated with a higher steroid sensitivity.
Cho HY et al. [43]	2009	Asian (Korea)	190/100	4.95/NA	No correlation between the INS onset age, initial steroid responsiveness, renal pathologic findings, or progression to end-stage renal disease and ER22/23EK, N363S, and <i>BclI</i> polymorphisms.
Teeninga N et al. [44]	2014	Caucasian (Holland)	113	4.1	Carriers of GR-9β + <i>TthIII</i> mutated haplotype had a significantly higher incidence of SD compared with non-carriers.
Ye J et al. [52]	2006	Asian (China)	138	7.1	No association found with the studied polymorphisms.

SD: steroid dependant; FR: frequent relapser; NFR: non frequent relapse; SS: steroid sensitive; SR: steroid resistant; NR: non responder

First author	Year	Ethnicity	Case/Control	Age (mean)	Results
Results for genetic analysis of pro- and anti-inflammatory mediators in the downstream signaling pathway of the GC-GR complex in INS patients					
IL-12					
Muller-Berghaus J <i>et al.</i> [8]	2008	Caucasian (Germany)	79	10.7	Significantly higher allele frequency of IL12Bpro-1 in steroid-dependent children compared to children without SD.
TNF					
Kim SD <i>et al.</i> [95]	2004	Asian (Korea)	152/292	NA/NA	No association with TNF and IL-1beta.
Jafar T <i>et al.</i> [96]	2011	Asian (India)	150/569 115(SS)/35(SR)	4.8/NA	Association for <i>TNFA</i> (G308A) comparing patient with controls and SR group with SS group.
Tripathi G <i>et al.</i> [97]	2008	Asian (India)	115(SS)/35(SR)	4.8	The AA genotype of <i>TNFA</i> (G308A) was associated with lower steroid response.
MIF					
Berdeli A <i>et al.</i> [105]	2005	Caucasian (Turkish)	214/103 137(SS)/77(SR)	3.5/NA	Significant increase in <i>MIF</i> G-173C GC genotype and C allele frequency in INS and higher frequency of CC genotype in the SR group.
Vivarelli M <i>et al.</i> [106]	2008	Caucasian (Italian)	257/355	5.8/NA	Frequency of <i>MIF</i> -173*C allele was higher in patients with INS than in controls and more frequent in SR patients compared with steroid responders.
Choi HJ <i>et al.</i> [107]	2011	Asian (Korea)	170/100	5.17/NA	No association with <i>MIF</i> G-173C.
Swierczewska M <i>et al.</i> [108]	2014	Caucasian (Poland)	71/30	10.1/10.1	<i>MIF</i> CT genotype of rs2070767C>T associated with the risk of SR, while the distribution of TG genotype of rs2000466T>G was higher in SS children compared to SR.
IL-4					
Jafar T <i>et al.</i> [96]	2011	Asian (India)	150/569	4.8/NA	Association for <i>IL-4</i> (C590T) polymorphism comparing patients with controls and SR group with SS group.
Tripathi G <i>et al.</i> [97]	2008	Asian (India)	115(SS)/35(SR)	4.8	The TT genotype of <i>IL-4</i> (C590T) polymorphisms associated with reduced steroid response.
Ikeuchi Y <i>et al.</i> [119]	2009	Asian (Japan)	85/127	NA	<i>IL-4R alpha</i> (Ile50Val) mutated genotype less frequent in patients with 4 or more relapses compared to those who experienced fewer recurrences.
IL-6					

First author	Year	Ethnicity	Case/Control	Age (mean)	Results
Jafar T <i>et al.</i> [96]	2011	Asian (India)	150/569	4.8/NA	Association for <i>IL-6</i> (G174C) comparing patient with controls and SR group with SS group.
Tripathi G <i>et al.</i> [97]	2008	Asian (India)	115(SS)/35(SR)	4.8	The GG genotype of <i>IL-6</i> (G174C) polymorphism associated with reduced steroid response.

SD: steroid dependant; FR: frequent relapser; NFR: non frequent relapse; SS: steroid sensitive; SR: steroid resistant; NR: non responder

First author	Year	Ethnicity	Case/Control	Age (mean)	Results
Results for P-gp expression analysis					
Wasilewska A <i>et al.</i> [141]	2006	Caucasian (Poland)	88/18	10.0/9.18	Expression of P-gp higher in SD and FR than in NFR.
Wasilewska A <i>et al.</i> [140]	2006	Caucasian (Poland)	18/18	5.75/6.50	Expression of P-gp higher in patients in relapse than in controls and decreased in remission.
Funaki S <i>et al.</i> [142]	2008	Asian (Japan)	14	10.4	mRNA levels decrease in complete remission in SS.
Stachowski J <i>et al.</i> [143]	2000	Caucasian (Poland)	39	(range 3-8)	Higher expression of P-gp mRNA in SR than in SS.
Prasad N <i>et al.</i> [68]	2015	Asian (India)	26/10	8.0/NA	Expression of P-gp higher at baseline and at the time of relapse compared to remission.
Results for genetic analysis of SNPs C1236T, G2677T/A, C3435T					
Wasilewska A <i>et al.</i> [148]	2007	Caucasian (Poland)	108/135	11.13/6.23	SNPs associated with time to response, TTT haplotype associated with late steroid response.
Choi HJ <i>et al.</i> [107]	2011	Asian (Korea)	170	5.17	Frequencies of 1236CC and CT higher in initial steroid responders than in NR, frequency of TGC haplotype lower in the initial steroid responders than in NR.
Jafar T <i>et al.</i> [149]	2011	Asian (India)	216/216	5.0/6.0	Frequency of 2677GG/AA higher in SR than in SS. Combination of 3435TT and 2677TT/AA increased the risk of SR.
Chiou YH <i>et al.</i> [150]	2012	Asian (Taiwan)	74	3.9(SS), 7.2(SR)	1236 T allele associate with SR.
Youssef DM <i>et al.</i> [151]	2013	African (Egypt)	138/140	2.7(SS), 4.6(SR)	Frequency of mutated and heterozygous G2677T/A higher in SR.
Cizmarikova M <i>et al.</i> [152]	2015	Caucasian (Slovakia)	46/100	6.42/7.89	3435TC was associated with SS.

SD: steroid dependant; FR: frequent relapser; NFR: non frequent relapse; SS: steroid sensitive; SR: steroid resistant; NR: non responder



