Adrenal insufficiency during treatment for childhood acute lymphoblastic leukemia is associated with glucocorticoid receptor polymorphisms ER22/23EK and *Bcl*I

Glucocorticoids play a major role in the treatment of acute lymphoblastic leukemia (ALL).[1,2] Supraphysiological doses of glucocorticoids may, however, suppress the hypothalamus-pituitary-adrenal (HPA) axis and consequently lead to an impaired stress response and an inadequate host defense against infections, which remains a cause of morbidity and death.[3] The duration of adrenal insufficiency after cessation of glucocorticoids is known to show considerable inter-individual variation.[4-6]

A large number of polymorphisms in the glucocorticoid receptor (GR) gene (NR3C1), which is located on chromosome 5, are known. The ER22/23EK polymorphism (rs6189 and rs6190, G>A, located on exon 2) and the GR-β polymorphism (rs6198, A>G, located on exon 9β) have both been associated with a relative glucocorticoid resistance.[7,8] In contrast, the N363S polymorphism (rs56149945, previously rs6195, A>G, located in codon 363 of exon 2) and the *Bcl*I polymorphism (rs41423247, C>G, located 646 nucleotides downstream from exon 2) have been reported to be associated with an enhanced sensitivity to glucocorticoids.[7,9] We performed a pilot study to explore the relation between the duration of adrenal insufficiency after a 4-week induction course with high-dose prednisone therapy (60 mg/m^2/day in three doses) with tapering over nine days and the incidence of GR polymorphisms with previously reported altered sensitivity for glucocorticoids in children treated according to the Dutch Childhood Oncology Group ALL 10 or the consecutive ALL 11 protocol.[10,11]

Adrenal function was assessed by the intravenous low-dose (1 μg) ACTH stimulation test using tetracosactrin (Synacthen, Novartis).[12,13] A normal response was defined as a stimulated serum cortisol of 550 nmol/L or over.[13] The first test was performed approximately one week after the last prednisone tapering dose according to the induction course. In case of adrenal suppression, the test was repeated every 2-3 weeks, until normal stimulated cortisol levels were observed or until the end of follow up which was 96 days after the last prednisone dose because of the start of a second high-dose glucocorticoid course.

DNA of the 25 included patients was extracted from leukocytes in peripheral venous blood samples taken during hematologic remission using standard techniques. Genotypes were determined by allelic discrimination using

Table 1. Genotype distribution in study sample.

	ER22/23EK			GR-β			N363S			*Bcl*I		
	Non-carrier	Carrier	*P* value	Non-carrier	Carrier*	*P* value	Non-carrier	Carrier	*P* value	Non-carrier	Carrier**	*P* value
Participants, N	23	2		16	9		24	1		12	13	
Age, years: median (range)	6.0 (1.0-16.0)	10.5 (5.0-16.0)	0.43	4.5 (1.0-16.0)	10.0 (4.0-16.0)	0.06	6.0 (1.0-16.0)	12.0 (NA)	NA	11.0 (1.0-16.0)	4.0 (1.0-13.0)	0.02
Sex, male (%)	44	50	1.00	38	56	0.43	42	0	NA	50	39	0.70
Treatment protocol			1.00			1.00			NA			0.32
ALL 10	19	2		13	8		20	1		9	12	
ALL 11	4			3	1		4			3	1	

**of which two patients are homozygous; **of which 4 patients are homozygous; NA: not applicable.*

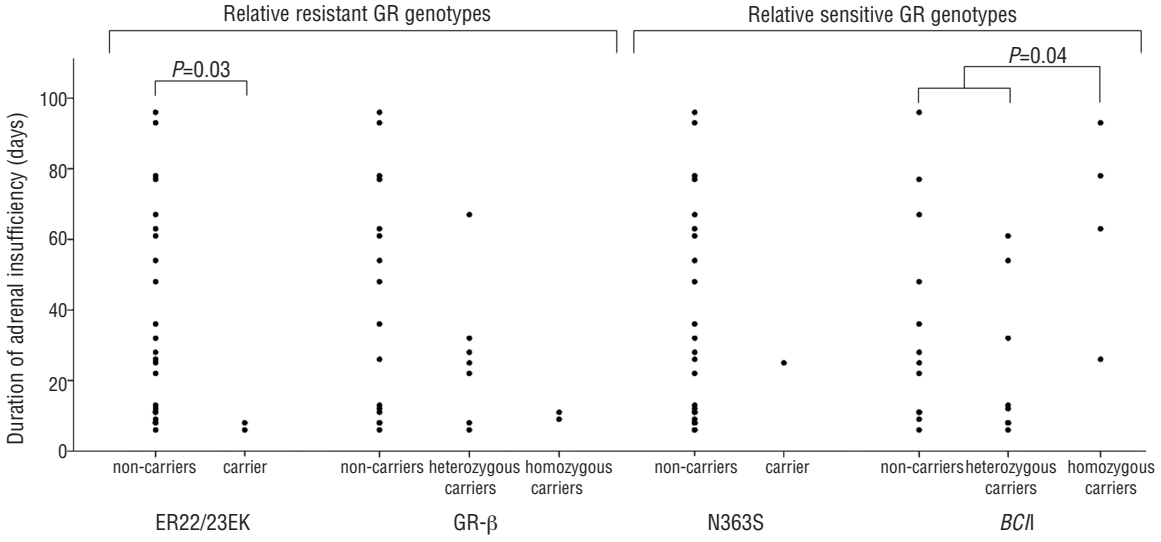

Figure 1. Duration of adrenal insufficiency after a 4-week predniso(lo)ne induction course in ALL patients with different GR genotypes.

a Taqman ABI Prism 7900HT Sequence Detection System. The Assay-by-Design service (*http://www.appliedbiosystems.com*) was used to set up a Taqman allelic discrimination assay.[14] Table 1 shows the GR genotype distribution; frequencies are similar to those reported previously.[15] There was no correlation between patient age and duration of adrenal insufficiency (r=0.24, *P*=0.24). Figure 1 shows the duration of adrenal insufficiency in childhood ALL patients after induction therapy with high-dose prednisone for the different GR genotypes. The duration of adrenal insufficiency in carriers of ER22/23EK was shorter than in the non-carriers (median 7.0 days (range 6.0-8.0) *vs.* 28.0 days (range: 6.0-96.0); *P*=0.03). There was no statistically significant difference in duration of adrenal insufficiency between carriers and non-carriers of GR-β (median 22.0 days (range 6.0-67.0) *vs.* 42.0 days (range 6.0-96.0) days; *P*=0.17) nor between patients homozygous for GR-β and non-carriers (median 10.0 days (range 9.0-11.0) vs. 42.0 days (range 6.0-96.0); *P*=0.21). Because there was only one carrier, the influence of the genotype N363S could not be statistically analyzed. The duration of adrenal insufficiency in patients homozygous for *Bcl*I was longer than in patients who were not homozygous for Bcl*I* (median 70.5 days (range 26.0-93.0) *vs.* 22.0 days (range 6.0-96.0); *P*=0.04).

In conclusion, the duration of adrenal insufficiency after discontinuation of a 4-week high-dose prednisone induction course is affected by GR genotype, which to the best of our knowledge has never been previously reported. The duration of adrenal insufficiency in carriers of the ER22/23EK polymorphism[7,8] was shorter than in non-carriers, indicating a lower susceptibility for developing adrenal insufficiency after high-dose glucocorticoid therapy. Adrenal insufficiency in patients homozygous for *BclI*[7,9] lasted longer in comparison to patients who were not homozygous for *Bcl*I. This indicates that patients homozygous for *Bcl*I have an increased susceptibility for prolonged adrenal insufficiency. A clear limitation of this pilot study is the limited number of patients included. Nevertheless, despite the small sample size and the relatively low frequencies of the GR polymorphisms in the general population, we observed statistically significant and clinically relevant associations between certain GR genotypes and the duration of adrenal insufficiency, which are biologically plausible. This indicates that the duration of adrenal insufficiency during treatment for childhood ALL can, at least partially, be attributed to variants of the GR gene that encodes for the protein through which glucocorticoids exert their action.

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doi:10.3324/haematol.2014.105056

Key words: adrenal insufficiency, ALL, childhood, GR polymorphisms.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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