Correspondence

To the editor:

Increased frequency of the glucocorticoid receptor A3669G (rs6198) polymorphism in patients with Diamond-Blackfan anemia

The human glucocorticoid receptor (GR) is encoded by GR/NR3C1 located in the 5q31-32 cytoband of chromosome 5, which is deleted in patients with 5q− myelodysplastic syndrome. The gene is highly polymorphic, containing single nucleotide polymorphisms (SNPs) both in the coding region and in regions associated with alternative splicing and mRNA stabilization. The most studied receptor is the GRα isoform, which, on binding to its ligand, migrates to the nucleus where it activates/suppresses expression of target genes. The combination of SNPs, alternative splicing, and posttranslational modifications generates >200 alternative GRα isoforms that differ widely in terms of ligand and/or DNA binding affinity. In addition, poorly defined mechanisms, possibly involving microenvironmental cues, activate alternative splicing of exon 9, which generates mRNA encoding the dominant-negative GRβ isoform. The A3669G (rs6198) SNP in the untranslated region of GRβ exon 9 stabilizes GRβ mRNA and is present with an allele frequency between 4% (Sub-Saharan Africans) and 20% (Europeans) in the normal population but at greater frequencies in patients with autoimmune disorders (27% in systemic lupus erythematosus and 42% in rheumatoid arthritis) and in individuals predisposed to central adiposity (30.4%). In this issue of Blood, we describe that the frequency of the A3669G SNP is increased in polycythemia vera (PV; 55%) and that erythroblasts expanded in vitro from these patients express GRβ and do not respond to corticosteroids. Based on these and other observations, we suggest that GRβ may represent a “host genetic modifier” that in combination with JAK2 mutations determines erythrocytosis.

Diamond Blackfan anemia (DBA), a rare congenital form of red cell aplasia, presents with profound aregenerative anemia in early infancy. In approximately half of the cases, mutations in genes encoding proteins present in either the small or large ribosomal subunits have been detected. Clinical management of DBA includes corticosteroid therapy and for the 40% of patients who, for reasons still to be understood, do not respond to corticosteroids, red cell transfusions and/or hematopoietic stem cell transplantation. The increased frequency of the A3669G SNP in PV patients whose erythrocytosis did not respond to corticosteroids in vitro prompted us to determine the frequency of this SNP in DBA patients. A total of 58 patients were analyzed, of whom 86% (n = 50) presented mutations in RPL5, RPL11, RPS19, and RPS26. For 8 patients, no mutation has been identified. Thirty-one patients presented with the de novo form of the disease, 10 the familial, 13 the sporadic, and 5 are unknown. The A3669G SNP was found in 25 (43%) of the 58 DBA patients compared with 70 (28.5%) of 246 nondiseased white donors (P = .03; Table 1). Although the frequency of the A3669G SNP was increased in patients carrying mutations in RPS19 (36%), the increase did not reach statistical significance (P = .053).

Recently, it has been described that p53 is overexpressed in hematopoietic cells from patients carrying mutations, leading to haploinsufficiency of the ribosomal proteins RPS19 (DBA) and RPS14 (5q− syndrome), and that pharmacologic inhibition of p53 rescues the defective erythroid maturation expressed by RPS19- or RPS14-deficient progenitor cells in vitro. These data, and the well-described ability of p53 to up-regulate GR expression, suggest that the A3669G SNP may represent a “host genetic modifier,” which may be the cause of incomplete penetrance in DBA patients. To clarify this point, we first assessed the relationship between presence of the SNP and glucocorticoid responsiveness. Of the 35 DBA patients for whom response to corticosteroids was known, 10 became transfusion-independent. There was a trend (P = .086) toward increased frequency of corticosteroid-responsive patients in the SNP negative group, of whom 50% were corticosteroid-responsive whereas only 17% of the SNP-positive patients responded to corticosteroids. However, based on the P value obtained on this preliminary observation, an 80% power analysis indicates that at least 50 patients per group, a total of 160 patients, would be required to clarify whether the presence of A3669G and steroid responsiveness are associated in DBA. Second, we compared the frequency of homozygosity for the G allele in DBA patients and nondiseased white donors. G allele homozygosity was higher in the patients (7%) compared with controls (2%), but all A3669G genotype frequencies were in Hardy-Weinberg equilibrium in both the DBA patients and nondiseased white donors. It is possible that G allele homozygosity generated in vivo by somatic recombination may provide a proliferative advantage to DBA hematopoietic stem/progenitor cells. It is also possible that the A3669G SNP is not directly

Table 1. Frequency of the A3669G (rs6198) polymorphism in nondiseased Caucasian donors and DBA patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal donors</th>
<th>DBA patients</th>
<th>P vs ND</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>176 (72)</td>
<td>33 (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>65 (26)</td>
<td>21 (36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>5 (2)</td>
<td>4 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/G + G/G</td>
<td>70 (28)</td>
<td>25 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>n.a.</td>
<td>.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>417 (85)</td>
<td>87 (75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>75 (15)</td>
<td>29 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>n.a.</td>
<td>.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are n (%) unless otherwise specified. DBA: DNA was prepared from mononuclear cells collected from 58 DBA patients at the time of their routine visit according to protocols approved by the University of Turin, Turin, Italy. Nondiseased donors: DNA from 246 anonymous nondiseased Caucasian donors was obtained from the Department of Genetics and Genomic Sciences of the Mount Sinai School of Medicine. All the samples were provided for this study as de-identified material and analyzed blindly for A3669G (rs6198). In the case of 44 DBA patients, the frequency of the SNP was detected by genomic PCR amplification followed by direct sequencing. All the DBA patients and the nondiseased donors were also genotyped by restriction fragment length polymorphism analyses using previously published amplification primers and Swal. Genotype distributions and allele frequencies were analyzed according to the Fisher exact test and y2, respectively. ND indicates nondiseased donors; and n.a., not applicable.
associated with steroid responsiveness but is in linkage disequilibrium with other SNPs, in either GR or another gene, which are in turn responsible for this phenotype. In this regard, given the high degree of genetic polymorphism at the GR locus, sequencing of the entire GR gene, coupled with biochemical characterization of the corresponding protein, will be necessary to determine whether additional polymorphisms in the coding region that alter the protein structure and/or transcriptional activity of GRβ may also affect steroid responsiveness or other clinical features of these patients.

In conclusion, these data identify the first genetic link between polymorphism of GR and the DBA phenotype, which may explain the variegation of phenotype observed in this patient population.

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Acknowledgments: This study was supported by a grant from the NY-STAR foundation (C-06066), New York, New York, and by institutional funds from the Istituto Superiore Sanità, Italy.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


To the editor:

Tolvaptan inhibition of desmopressin effects on coagulation factors in a patient with decreased von Willebrand factor and polycystic kidney disease

Desmopressin (dDAVP) is a synthetic analog of vasopressin that stimulates release of von Willebrand factor (VWF). This effect is attenuated in vitro by a type 2 vasopressin receptor (V2R) antagonist.1 dDAVP is administered for prophylaxis and treatment of bleeding in von Willebrand disease (VWD), mild hemophilia, and chronic kidney disease (CKD).

In autosomal dominant polycystic kidney disease (ADPKD), cyclic AMP promotes abnormal growth and proliferation of renal epithelium that results in cyst formation and CKD.2,3 V2R inhibition attenuates progression of CKD in rodent orthologs of ADPKD.4 Tolvaptan, a V2R antagonist, is effective treatment for hyponatremia and is being evaluated in clinical trials as treatment for ADPKD.4,5

Tolvaptan’s effects on dDAVP stimulation of VWF have not been reported. We evaluated a 42-year-old man with ADPKD found to have low VWF levels during screening for a study of tolvaptan treatment for ADPKD. A comprehensive bleeding history, using a standardized instrument,6 found no excessive bleeding with several minor procedures, but revealed one episode of hematuria from renal cyst hemorrhage and one episode of hemoptysis during an upper respiratory infection. Laboratory tests (Table 1)
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