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A novel human glucocorticoid receptor SNP results in increased transactivation potential☆

Tajia L. Green, Kelly Tung, Debora Lim, Stacey M. Leventhal, Kiho Cho, David G. Greenhalgh*

Shriners Hospitals for Children Northern California, and Department of Surgery, University of California, Davis, Sacramento, CA 95817, USA

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ABSTRACT

Glucocorticoids are one of the most widely used therapeutics in the treatment of a variety of inflammatory disorders. However, it is known that there are variable patient responses to glucocorticoid treatment; there are responders and non-responders, or those that need higher dosages. Polymorphisms in the glucocorticoid receptor (GR) have been implicated in this variability. In this study, ninety-seven volunteers were surveyed for polymorphisms in the human GR-alpha (hGRα), the accepted biologically active reference isoform. One isoform identified in our survey, named hGR DL-2, had four single nucleotide polymorphisms (SNPs), one synonymous and three non-synonymous, and a four base pair deletion resulting in a frame shift and early termination to produce a 743 amino acid putative protein. hGR DL-2 had a decrease in transactivation potential of more than 90%. Upon further analysis of the individual SNPs and deletion, one SNP, A829G, which results in a lysine to glutamic acid amino acid change at position 277, was found to increase the transactivation potential of hGR more than eight times the full-length reference. Furthermore, the hGRα-A829G isoform had a differential hyperactive response to various exogenous steroids. Increasing our knowledge as to how various SNPs affect hGR activity may help in understanding the unpredictable patient response to steroid treatment, and is a step towards personalizing patient care.

1. Introduction

Glucocorticoids are steroid hormones that regulate a variety of biological processes including stress response, glucose metabolism, cellular differentiation, and inflammation and immune response [1,2]. They are released from the adrenal cortex in response to trauma, pathogens, as well as other types of physiological and psychological stress in an attempt to return the body to homeostasis [3,4]. The human endogenous glucocorticoid is cortisol. There are a wide range of diseases that are treated with glucocorticoids: asthma, rheumatoid arthritis, Graves' disease, ulcerative colitis, and sepsis [5,6]. Their potency and diverse effects have made them one of the most widely prescribed drugs in the world [3]. However, response to glucocorticoid treatment can also vary greatly between patients; some require more aggressive treatments at higher doses, some smaller doses, while some appear to be glucocorticoid resistant [7–12].

Glucocorticoids are reported to act by binding to the glucocorticoid receptor (GR), a cytoplasm-localized receptor belonging to the nuclear steroid receptor superfamily [1]. Like the other members of the family, GRs are known to be comprised of an N-terminal transactivation domain, DNA binding domain, and a C-terminal ligand binding domain [5]. The human reference GR (hGR) has nine exons, of which only exons two through nine are translated into a protein (Fig. 1). Exon nine is alternatively spliced to form either hGRα or hGRß. hGRα is the classical GR isoform [13,14]. In the classical GR pathway, upon binding glucocorticoid, hGRα dissociates from its chaperone proteins and translocates to the nucleus where it activates or represses various genes directly by binding to specific transcription regulatory elements known as the glucocorticoid response element (GRE) and negative GRE (nGRE), or indirectly by tethering to other transcription factors [13–16]. hGRß acts as a dominant negative inhibitor of hGRα; however, hGRß is able to bind the glucocorticoid antagonist RU486, and is also able to modulate gene transcription independently of hGRα [17,18].

As a key factor in mediating the glucocorticoid response, variations in the GR have been widely studied. The overwhelming majority of hGR polymorphisms are connected with loss of function and often glucocorticoid resistance [19,20]. There have been relatively few gain-of-function GR polymorphisms reported and most were generated in the laboratory [21–23]. However, Tung et al. identified a naturally occurring hyperactive hGR resulting from a combination of three

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* Correspondence to: Shriners Hospitals for Children Northern California, 2425 Stockton Blvd., Sacramento, CA 95817, USA.
E-mail addresses: tlvanhook@ucdavis.edu (T.L. Green), dllim@ucdavis.edu (D. Lim), smleventhal@ucdavis.edu (S.M. Leventhal), kcho@ucdavis.edu (K. Cho), dggreenhalgh@ucdavis.edu (D.G. Greenhalgh).

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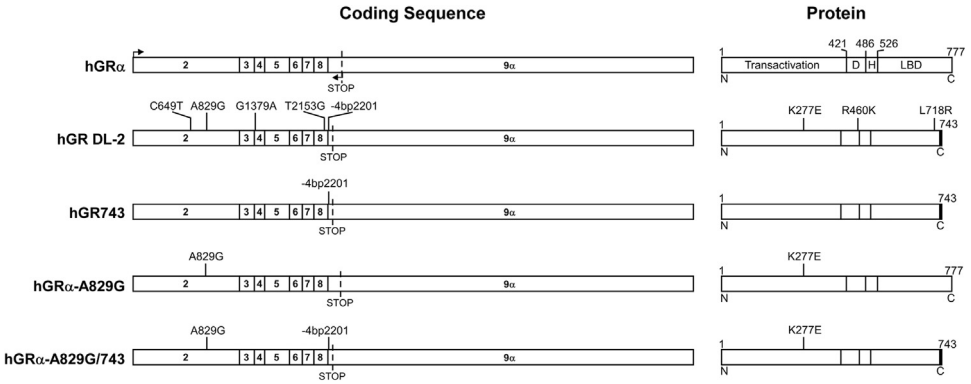

Fig. 1. *Coding sequences and protein structure of hGR isoforms.* Reference hGR is comprised of nine exons, of which exons two through nine are translated. hGRα, the reference, is listed first. The start position is indicated with an arrow. hGRα is followed by hGR DL-2 and its derivative isoforms. Based on the coding sequence, SNPs and deletions are indicated on each isoform. The position of the stop codon for each isoform is marked by a dashed vertical line. Adjacent to each coding sequence is the putative protein. hGRα is translated into a 777 amino acid protein which is made of a transactivation domain, DNA binding domain (D), hinge region (H), and ligand binding domain (LBD). The boundaries of each region are indicated. The amino acid changes of hGR DL-2 and derivatives are noted for each putative protein. The frame shift caused by the four base pair (bp) deletion results in the last ten amino acids differing from reference hGRα (black box).

non-synonymous single nucleotide polymorphisms (SNPs): A214G, T962C, and A2297G [24].

While screening various human subjects for GR polymorphisms, we identified an isoform, hGR DL-2, that has one synonymous and three non-synonymous SNPs and a four base pair deletion at position 2201 of the coding sequence. This caused a frame shift and early termination resulting in a 743 amino acid putative protein (Fig. 1). This isoform had negligible activity. However, when each individual SNP was isolated and tested for activity, we found that one SNP, A829G (K277E) displayed a hyperactive response relative to hGRα. Identifying alterations such as this may contribute to a greater understanding of the variable response to glucocorticoid treatment.

2. Materials and methods

2.1. Study population

The details of the study population have been previously described [24]. In brief, this study was approved by the institutional review board of the University of California, Davis, and all participants gave informed written consent. Excluded from the study were those with major medical conditions, such as diabetes mellitus, hypertension, chronic obstructive pulmonary disease, inflammatory bowel disease, autoimmune diseases, cancer, pregnancy or exogenous steroid regimens. The study cohort consisted of 97 volunteers (70 female and 27 male; 20–67 years of age at the time of blood collection).

2.2. Identification, construction, and nomenclature of hGR isoforms

Total RNA was isolated from the buffy coat using the RNeasy Mini Prep kit with a modified protocol (Qiagen, Valencia, CA) followed by reverse transcription with Sensiscript RT (Qiagen). Subsequently, the hGR coding sequence was amplified by polymerase chain reaction in two sections: exons 2 to 3 (hGR-1B: tcactgatggactccaaag; hGR 3-2A: aagcttcatcagagcacacc) and exons 3 to 9α (hGR 3-1A: ccagcatgagaccagatgta; hGRa-2A: ttaaggcagtcacttttgatgaaac). Each section was cloned into the pGEM-T Easy vector (Promega, Madison, WI) and sequenced at MC Laboratories (South San Francisco, CA). Sequences were compared to the hGRα reference sequence from National Center for Biotechnology Informatics (NCBI) (NM_001018077) to identify polymorphisms. A full-length coding sequence was created by combining the fragments after cutting with restriction enzymes, then sub-cloning into a pcDNA4-HisMax expression vector (Life Technologies, Grand Island, NY).

Using the hGR DL-2 and hGRα isoforms as templates, each of the SNPs and the deletion were individually separated into new constructs, and then each SNP was paired with the deletion in derivative isoforms using a schema of restriction digests so that the action of each could be studied independently.

hGR isoforms are named based on structure. hGRα refers to an isoform that matches the NCBI hGRα reference sequence (NM_001018077). hGR DL refers to an isoform with a deletion (DL) followed by a number indicating the sequence in which our laboratory examined the deletion isoform. Derivative isoforms isolating individual SNPs are designated by hGRα followed by their SNP change (position with nucleotide change based on coding sequence). hGR743 has no SNPs and is named based on the resulting putative protein size.

2.3. Measurement of transactivation potential of hGR isoforms

tsA201 cells (a HEK 293 cell subclone stably transfected with the SV40 large T-antigen) were a gift from Dr. Daniel Feldman (Shriners Hospitals for Children Northern California). For each transfection, tsA201 cells were seeded on a 96-well plate at either 20,000 cells per well (baseline, no steroid stimulation assay) or 12,000 cells per well (vehicle and steroid stimulation assay) in 100 µl of antibiotic-free Dulbecco's Modified Eagle Medium (Life Technologies) supplemented with 10% fetal bovine serum (JR Scientific, Woodland, CA or Atlanta Biologicals, Lawrenceville, GA) and incubated at 37 °C with 5% CO_2. The next day the cells were transfected with an hGR isoform and a glucocorticoid response element (GRE)-luciferase reporter plasmid (PathDetect GRE Cis-Reporter Plasmid; Agilent Technologies, La Jolla, CA) using Fugene 6 (Promega) per the manufacturer's protocol. The following day, for baseline assays, the transactivation potential of the cells was assessed; otherwise cells were treated with graded doses of hydrocortisone (1–100 nM), methylprednisolone (10^{-3} nM to 1 nM), dexamethasone (10^{-4} nM to 1 nM), or vehicle control. For hydrocortisone and methylprednisolone 0.9% saline was the vehicle, and for dexamethasone a solution consisting of 1% benzyl alcohol, 1.1% sodium citrate, and 0.1% sodium sulfite in water was the vehicle. The concentration ranges were determined based on previous titration studies to optimize the response of hGR to each steroid (data not shown). Pharmaceutical-grade hydrocortisone sodium succinate (Pfizer, New York, NY; clinical anti-inflammatory adult dosage, 15–240 mg; half-life, 8–12 h), methylprednisolone sodium succinate (Pfizer; clinical anti-inflammatory adult dosage, 10–40 mg; half-life, 12–36 h), and dexamethasone sodium phosphate (Luitpold Pharmaceuticals, Shirley, NY; clinical anti-inflammatory adult dosage, 0.4–6 mg; half-life, 36–72 h) were used. A Luciferase Assay Kit (Agilent Technologies) was used to determine the transactivation potential of each isoform and luminescence was measured with a

Perkin-Elmer MicroBeta Trilux (Perkin-Elmer, Waltham, MA).

2.4. Western blot analysis of hGR isoforms

tsA201 cells transfected with recombinant hGR isoforms were either lysed in ice-cold lysis buffer (Agilent) supplemented with cOmplete Protease Inhibitor Cocktail (Roche, Indianapolis, IN) and supernatants harvested and normalized, or were fractionated into nuclear, cytoplasmic, and membrane extracts using a cell fractionation kit (Cell Signaling Technology, Danvers, MA). Proteins were run on a 4–20% BioRad Criterion TGX gel (Hercules, CA), then transferred to a polyvinylidene difluoride membrane (BioRad). Membranes were blocked with 5% nonfat dry milk, washed, and incubated overnight with Glucocorticoid Receptor (D8H2) XP (1:1000), MEK1/2 (D1A5) (1:1000), AIF (D39D2) XP (1:1000), or Histone H3 (D1H2) XP (1:2000) rabbit monoclonal antibodies (Cell Signaling Technology) in 5% nonfat milk or bovine serum albumin. A secondary anti-rabbit-HRP (GE Healthcare, Piscataway, NJ) in 5% nonfat milk at 1:2000 was used for protein visualization via chemiluminescence using the ECL Prime Western Blot Detection System (GE Healthcare).

2.5. Statistical analysis

All luciferase assays were run in triplicate, except for vehicle controls which were run in duplicate. Each experiment was repeated at least three times to confirm the pattern, and then the data from multiple experiments were combined and normalized for figures and statistical analysis. The data are presented as means with error bars representing standard error of the mean. The results were compared with one-way ANOVA, and the significance was confirmed with a Tukey's post hoc test.

3. Results

3.1. Identification and transactivation potential of a novel hGR isoform and its derivatives

The survey of the study population for hGR polymorphisms identified numerous hGR isoforms with novel SNPs. One isoform chosen for further analysis because it also contained a deletion was hGR DL-2. hGR DL-2 had one synonymous (C649T) (rs78063502) and three novel non-synonymous SNPs (A829G, G1379A, T2153G) resulting in respective amino acid changes at positions 277 (transactivation domain), 460 (DNA binding domain), and 718 (ligand binding domain). It also had a four base pair deletion at position 2201 of the coding sequence (Fig. 1), causing a frame shift and early termination resulting in 743 amino acids versus the 777 amino acids in hGRα, the reference hGR. The last 10 amino acids of hGR DL-2 also differs from the reference. Functional analysis of hGR DL-2 found that this isoform had a more than 90% decrease in activity compared to hGRα (Fig. 2).

To further understand the effect of the variations in hGR DL-2, nine new constructs were made using hGRα as the base: one for each of the SNPS (C649T, A829G, G1379A, T2153G) singly, one for each of the SNPs in conjunction with the four base pair deletion, and one with only the four base pair deletion (Fig. 1, Supplementary Fig. 1). We found that the isoform with only the deletion, named hGR743 because of the resulting truncated protein size, had the same 90% loss in activity as hGR DL-2 (Fig. 2). The activity of the other SNP isoforms varied. C649T, the synonymous SNP, and G1379A had no significant effect on activity compared to hGRα; whereas T2153G, which changed leucine (L) to arginine (R) at position 718 in the ligand binding domain, resulted in a significant loss of activity (data not shown). The most interesting change, however, was the A829G SNP where lysine (K) became glutamic acid (E) at position 277 in the transactivation domain and resulted in an isoform that had more than eight times the activity of hGRα in the absence of steroid stimulation. Despite the significant increase in activity caused by the presence of A829G, this response was lost when the SNP was paired with the four base pair deletion. A Western blot confirmed the protein expression of the hGR isoforms as well as the resultant decrease in size of the truncated isoforms (Fig. 3A). Additionally, we looked at the nuclear, cytoplasmic, and membrane localization of hGRα-A829G in comparison to hGRα by Western blot (Fig. 3B). Blots for MEK1/2, AIF, and Histone H3 confirmed that protein expression was relatively even in the cytoplasmic, membrane, and nuclear fractions, respectively. We found that hGRα and hGRα-A829G was expressed in all three subcellular compartments while the reverse orientation hGRα negative control had no expression. Furthermore, hGRα-A829G did not have greater nuclear translocation than hGRα despite having a greater transactivation potential; in fact, the expression of hGRα-A829G was less than hGRα in all three fractions.

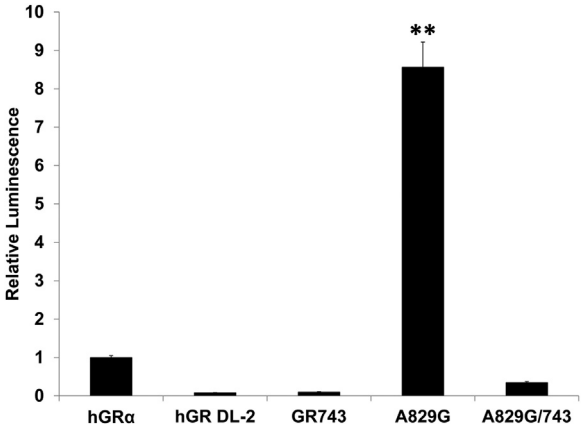

Fig. 2. *Baseline transactivation potential of hGR isoforms.* Without the addition of exogenous steroids there was a decrease in activity of all truncated isoforms, hGR DL-2, hGR743, and hGRα-A829G/743 versus hGRα. However, there was a significant increase in the activity of hGRα-A829G (**, $p < 0.01$) compared to all other isoforms. Data shown is a combination of seven experiments and presented as mean ± SEM.

3.2. hGR isoforms' differential responses to exogenous steroids

Subsequently, we looked at the response of hGRα-A829G to exogenous steroids. Initially, the constructs were treated with graded doses of hydrocortisone (1, 10, or 100 nM). Our previous studies have

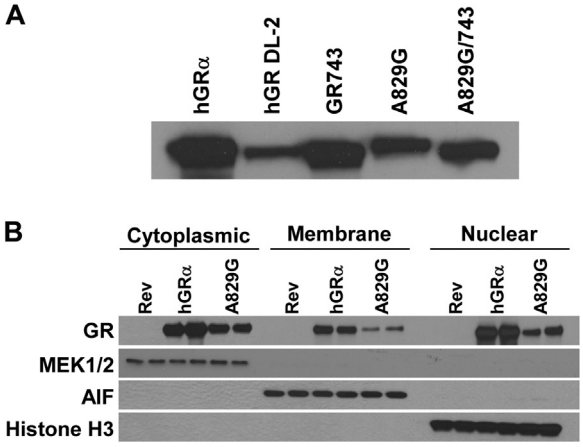

Fig. 3. *Expression and subcellular localization of hGR isoforms.* (A) A Western blot for GR confirmed the expression of all isoforms. (B) hGRα, hGRα-A829G, and reverse orientation hGRα negative control were separated into cytoplasmic, membrane, and nuclear subcellular fractions and the localization of GR was determined by Western blot. hGRα had the greatest expression in all three groups. Efficient fractionation of subcellular compartments was confirmed by the relatively even expression of MEK1/2 for cytoplasm, AIF for membrane, and Histone H3 for nuclear.

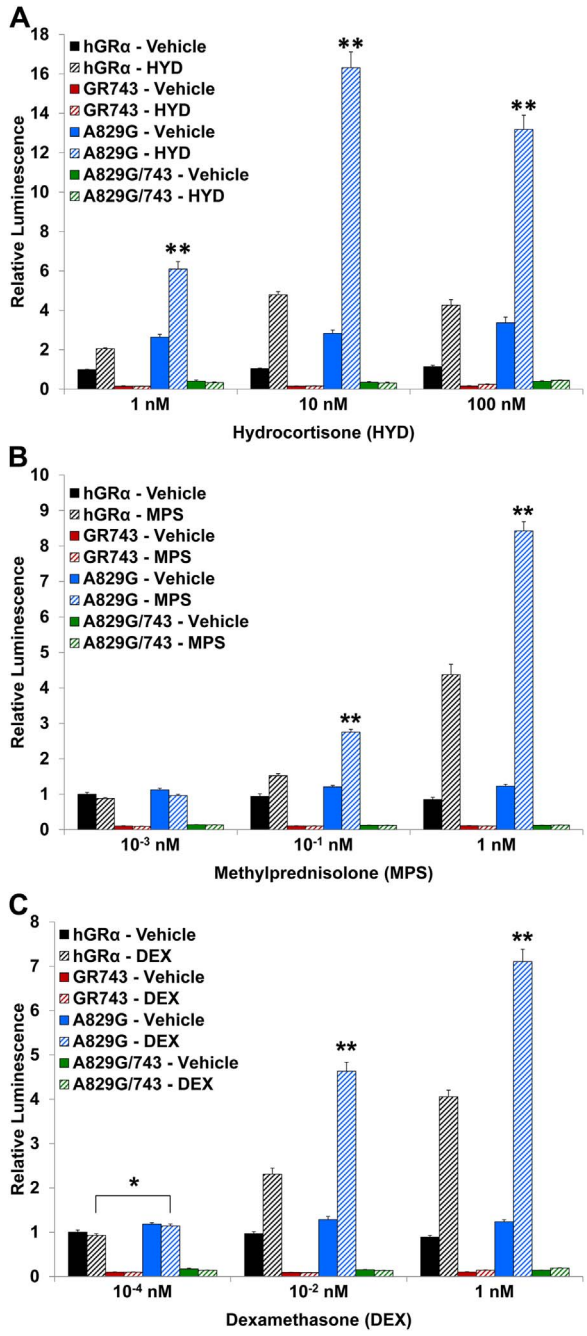

Fig. 4. *Transactivation potential of hGR isoforms with exogenous steroids.* (A) When stimulated with hydrocortisone, hGRα and hGRα-A829G had a dose dependent response that peaks at 10 nM of hydrocortisone. The activity of hGRα-A829G with hydrocortisone was significantly greater than all other isoforms at all concentrations (**, $p < 0.01$). Even after hydrocortisone stimulation, hGR743 and hGRα-A829G/743 had almost no activity. Data shown is a combination of three experiments and presented as mean ± SEM. (B) When stimulated with methylprednisolone, hGRα and hGRα-A829G had a dose dependent response that peaked at the highest concentration, 1 nM. The activity of hGRα-A829G with methylprednisolone is significantly greater than the other isoforms at 10^{-1} nM and 1 nM (**, $p < 0.01$); however, at the lowest concentration (10^{-3} nM) there is no difference in the activity of hGRα and hGRα-A829G. hGR734 and hGRα-A829G/743 again failed to significantly respond. Data shown is a combination of three experiments and presented as mean ± SEM. (C) hGRα and hGRα-A829G had a dose dependent response to dexamethasone stimulation which peaked at 1 nM. At the lowest concentration of dexamethasone, hGRα-A829G had greater activity than hGRα (*, $p < 0.05$). hGRα-A829G also had significantly greater activity than all other constructs at the higher concentrations of dexamethasone (10^{-2} and 1 nM) (**, $p < 0.01$). hGR743 and hGRα-A829G/743 also had no substantial activity when stimulated with dexamethasone. Data shown is a combination of three experiments and presented as mean ± SEM. HYD: hydrocortisone, MPS: methylprednisolone, DEX: dexamethasone.

shown that hGRα has a dose dependent response to hydrocortisone that peaks at ~10 nM [25,26] which was again confirmed in these studies (Fig. 4A). hGRα-A829G had a matching dose dependent response to hydrocortisone treatment which also peaked at 10 nM. However, hGRα-A829G had approximately three times the activity of hGRα at all concentrations tested. The other constructs containing the deletion, hGR743 and hGRα-A829G/743, still had no significant activity despite hydrocortisone stimulation.

hGRα and hGRα-A829G again had similar responses when stimulated with graded doses of exogenous methylprednisolone (10^{-3}, 10^{-1}, or 1 nM). Both constructs had a dose dependent response that peaked at 1 nM of methylprednisolone (Fig. 4B). At the two higher concentrations, 10^{-1} nM and 10^{-3} nM, the activity of A829G was still significantly greater than hGRα, but had only about twice the activity. Interestingly, at the lowest concentration, 10^{-3} nM of methylprednisolone had a slight negative effect on hGRα-A829G and decreased the activity so that there was no difference between hGRα and hGRα-A829G. Similar to the results seen in hydrocortisone, the hGR743 and hGRα-A829G/743 deletion isoforms had almost no activity when treated with methylprednisolone.

Lastly, the response of the hGR isoforms to dexamethasone was examined. They were treated with 10^{-4}, 10^{-2}, or 1 nM of dexamethasone. The results to dexamethasone were very similar to the results to methylprednisolone (Fig. 4C). Both hGRα and hGRα-A829G had a dose dependent response that peaked at the highest concentration, 1 nM. And again, the activity of hGRα-A829G was about two times the activity of hGRα at the two higher concentrations of steroid, 10^{-2} and 1 nM. However, unlike methylprednisolone, at the lowest concentration of dexamethasone, there was a difference in activity between hGRα-A829G and hGRα. The difference was not as great as the other concentrations, with only about 1.2 times the activity of hGRα. Also, as seen previously, hGR743 and hGRα-A829G/743 had no significant activity even when stimulated with exogenous dexamethasone.

3.3. hGR alterations near A829G

The A829G SNP was found in only one subject in our study population of 97 volunteers. The small size of our study population precludes any frequency analysis. Alternatively, as a follow-up, the 1000 Genomes Project database was surveyed to determine if A829G had been observed in other individuals and to identify any alterations in the immediate vicinity of positon 829 as well. We were unable to find A829G in the 1000 Genomes Project database; however, one deletion and 14 SNPs at 11 positions were identified in a 60 base pair region surrounding position 829 (Table 1). Half of the SNPs identified were synonymous. Three of the SNPs, A799G, A840T, and A846C, were also found in our study population. The deletion was a six base pair, in-frame deletion at positions 838–843 which removed a lysine and glutamine.

Table 1
SNPs adjacent to A829G in 1000 genomes database.

A799G[a]	Serine to Glycine
C801T	Synonymous
C804T	Synonymous
C804G	Synonymous
C804A	Synonymous
T807G	Serine to Arginine
G811A	Valine to Isoleucine
A836G	Glutamic acid to Glycine
A840T[a]	Glutamine to Aspartic acid
A840G	Synonymous
T846C[a]	Synonymous
A850G	Isoleucine to Valine
C856T	Leucine to Valine
C858A	Synonymous

[a] Also found in the volunteer population for this study.

4. Discussion

A829G (K277E) was one of many SNPs identified from the volunteer population. Currently, over 3000 SNPs have been identified in hGRα overall [27]. An overwhelming majority of the variations identified have a repressive effect, such as decreased transactivation potential and glucocorticoid resistance [19,28–30]. For example, a V423A SNP (T1268C) occurring in the DNA binding domain was found in a patient with Primary Generalized Glucocorticoid Resistance or Chrousos syndrome, a disease characterized by generalized, partial, end-organ insensitivity to glucocorticoids resulting in increased circulating levels of adrenocorticotropic hormone (ACTH) and cortisol [31,32]. Analysis of the V423A isoform showed that it displayed decreased DNA binding efficiency, a 72% reduction in transactivation potential, and delayed nuclear translocation [33]. A second SNP, G2035A, designated G679S, in the ligand-binding domain, was associated with primary glucocorticoid resistance and also had a significant decrease in transactivation potential accompanied by an approximate 50% decrease ligand binding affinity [34].

Far fewer hyperactive or glucocorticoid sensitive hGR isoforms have been identified. The most well-known, naturally occurring glucocorticoid sensitive variant is N363S (A1220G), which has been linked with a higher body mass index and increased insulin response to dexamethasone [35–37]. More recently, another naturally occurring, glucocorticoid sensitive variant caused by an SNP, D401H (G1201C), was identified [38]. The patient also presented with type 2 diabetes and visceral obesity, as well as hypertension. Additionally, during a study of the hGR ligand binding domain, Warriar et al. experimentally generated two variants, M565R (T1694G) and A573Q (GC1717CA), which were found to be hyperactive [21]. Our group also identified a hyperactive hGR isoform, hGR NS-1, from the same study cohort presented here [24]. The transactivation potential of hGR NS-1 was more than twice that of the reference hGRα. However, the activity of hGR NS-1 was a result of a combination of three SNPs (A214G, T962C, and A2297G) compared to the single SNP of hGRα-A829G, which results in eight times the activity of hGRα.

Three transactivation domains (τ1, τ2, and AF-2) and three sumoylation sites (K277, K293, K703) have been identified in hGR (Fig. 5) [39–42]. The A829G SNP exchanges the basic amino acid lysine to an acidic glutamic acid at position 277, the first sumoylation site. This position is directly downstream of the τ1 transactivation domain, spanning amino acids 77–262 [40,43]. When Hollengerg et al. removed a group of amino acids from 262 to 404, which contains the K277, hGR activity was not affected [40]. However, when examining the effect of sumoylation, a K277R construct was made which exchanges lysine for a comparable basic arginine. This construct had a 2.8 fold increase in transactivation compared to reference hGR after dexamethasone stimulation when assayed with an ARE (androgen response element)-luciferase reporter; there was no difference in activity without dexamethasone [39]. Additionally, microarray analysis showed that altering all three sumoylation sites (K→R) increased the number of genes regulated by dexamethasone compared to reference hGR [44]. Therefore, in examining hGRα-A829G, the removal of the repressive sumoylation site in combination with changing from a positive amino acid to a negative one (K277E) so close to the critical, strongly acidic τ1 domain may cause a change in the conformational shape of hGR in this region, resulting in a significant increase in transactivation potential by A829G, with and without steroid stimulation. The exact mechanism driving the increase in activity is unknown. Increased nuclear translocation of hGRα-A829G is not the driving force behind the increase in transactivation potential, as might be expected when considering the classical GR pathway. The overall reduced expression of hGRα-A829G in comparison to hGRα indicates that there may be an alternative method that is intrinsic to the hGRα-A829G isoform such as increased DNA binding affinity. Further studies will be needed to determine the exact mechanism.

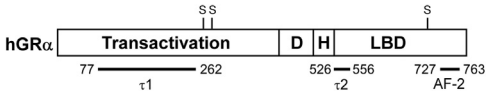

Fig. 5. *Diagram of hGR functional regions.* The sumoylation sites and the τ1, τ2, and AF-2 transactivation domains of the reference hGR are illustrated with their amino acid positions. S: sumoylation.

Conversely, the deletion of four bases beginning at position 2201 in hGR743 causes a frame shift and early termination in which the last ten amino acids (734–743) differ from reference hGRα, resulting in a significant loss of transactivation potential. The deletion in hGR743 causes an alteration and truncation to the carboxy-terminal AF-2 transactivation domain (727–763) which is part of the essential twelfth amphipathic α-helix of the ligand binding domain [42,45]. The AF-2 is conserved among nuclear receptors and interacts with coactivators [45–47]. hGR743 is similar in size and structure to hGRß, the most common alternative hGR splice variant. hGRß has 742 amino acids, of which the last 15 are unique compared to hGRα [48]. In addition to helix 12 and AF-2, hGRß is also missing helix 11. hGRß is also inactive and considered to be a dominant-negative inhibitor of hGRα [49]. Yudt et al. showed that the loss of the twelfth helix accounts for the loss of transactivation potential in hGRß, but that specific amino acids, K733 and P734, were responsible for the dominant-negative activity [50]. Therefore, although hGR743 retains helix 11, the loss of helix 12 likely accounts for the loss of activity for which the A829G SNP is unable to compensate for in the hGRα-A829G/743 isoform [51]. Furthermore, although the amino acid sequence of hGR743 diverges from hGRα at position 734, it is likely not to have the dominant-negative activity of hGRß because hGR743 has a leucine (K) and isoleucine (I) at positions 733 and 734, respectively, and the dominant-negative activity of hGRß is dependent on specific amino acids K733 and P734 at those positions [50].

The exact mechanism for the hyperactivity of hGRα-A829G is unknown. However, the identification of a novel SNP that is drastically able to alter the transactivation potential of hGRα is significant. The K277E amino acid change of hGRα-A829G removes a sumoylation site and lies just outside of the N-terminal τ1 transactivation domain; the core region in τ1 necessary for transactivation has been localized to amino acids 187–227 [52]. The change caused by A829G at position 277 supports the role of sumoylation on hGR function and suggests that τ1 can be significantly influenced by surrounding amino acids. Furthermore, the overall transactivation domain may need further refinement to account for interactions with other residues. This will be particularly significant when considering the number of SNPs near position 829 identified in the 1000 Genomes Project database. The function of these alterations is yet unknown; however, the data presented here shows that single point mutations can significantly affect hGR function in relation to type and dosage of steroid, and may clinically influence a patient's glucocorticoid sensitivity or resistance. As such, identifying a patient's glucocorticoid receptor profile may be an essential step in tailoring their glucocorticoid therapy to achieve the maximal therapeutic response while minimalizing the negative effects.

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Appendix A. Transparency document

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.12.003.

Appendix B. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.12.003.

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