FUNCTIONAL CHARACTERIZATION OF A NOVEL HETEROZYGOUS POINT MUTATION IN THE HUMAN GLUCOCORTICOID RECEPTOR GENE CAUSING PRIMARY GENERALIZED GLUCOCORTICOID RESISTANCE

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INTRODUCTION

Primary Generalized Glucocorticoid Resistance (PGGR) is a rare genetic condition caused by mutations in the human glucocorticoid receptor (hGR) gene, which alter hGR action and reduce tissue sensitivity to glucocorticoids. A new case of PGGR caused by a novel heterozygous point mutation in the *hGR* gene, which resulted in threonine (T) to isoleucine (I) substitution at amino acid position 556 in the ligand-binding domain of the receptor, has been recently reported in a patient with adrenal incidentaloma (**1**).

OBJECTIVE AND HYPOTHESES

To delineate the molecular mechanisms of action of the natural mutant receptor hGRαT556I.

METHODS

Plasmids

The plasmids used in this study included pRShGRα, pMMTV-luc, pGL4.73[hRluc/SV40], pF25GFP-hGRα, pRSVC(p50)-NF-κB, pRSVC(p65)-RelA and p(IκB)3-luc. The plasmids pRShGRαT556I and pF25GFP-hGRT556I were constructed by introducing the T556I mutation into the pRShGRα and pF25GFP-hGRα plasmids, respectively, using PCR-assisted site-directed mutagenesis.

Transactivation and Transrepression Assays

CV-1 cells were transiently transfected with pRShGRα or pRShGRαT556I (0,05μg/well), pMMTV-luc (0,5μg/well) and pGL4.73[hRluc/SV40] (0,1 μg/well) (for transactivation assays) or pRSVC(p50)-NF-κB (0.0125 μg/ well), pRSVC(p65)-RelA (0.0125 μg/well), and p(IκB)3-luc (0.125 μg/well) (for transrepression assays) using lipofectamine. Forty-eight hours later, cells were exposed to increasing concentrations of dexamethasone for 24 hours. Firefly and renilla luciferase activities were determined in the cell lysates.

Dexamethasone-Binding Assays

COS-7 cells were transiently transfected with pRShGRα or pRShGRαT556I (1.5μg/well) using lipofectamine. Confluent cells were incubated with 6 different concentrations of [^{3}H]-dexamethasone at 37° C in the presence or absence of a 500-fold molar excess of nonradioactive dexamethasone for 1 hour. Dexamethasone-binding assays were performed as previously described (**2**).

Nuclear Translocation Studies

HeLa cells were transfected with pF25GFP-hGRα or pF25GFP-hGRT556I (2μg/dish) using FuGENE 6. Sixteen hours later, cells were exposed to dexamethasone (10^{-6} M), and fluorescence was detected sequentially by an inverted fluorescence microscope.

RESULTS

Compared with the wild-type receptor (hGRαWT), the mutant receptor hGRαT556I demonstrated a 50% reduction in its ability to transactivate the glucocorticoid-inducible MMTV promoter in response to dexamethasone (**Figure 1A**), and did not exert a dominant negative effect upon the hGRαWT (**Figure 1B**). Transrepression assays showed that the hGRαT556I displayed enhanced ability to transrepress the NF-κB signaling pathway in response to increasing concentrations of dexamethasone **(Figure 1C).** In dexamethasone-binding assays, the affinity of the mutant receptor hGRαT556I for the ligand was 2-fold lower than that of the hGRαWT (21.3 ± 4.09 nM *vs.* 10.8 ± 0.99 nM) (**Figure 1D**). In subcellular localization and nuclear translocation studies, both the hGRαWT and hGRαT556I were predominantly localized in the cytoplasm of cells in the absence of ligand. Addition of dexamethasone resulted in slower translocation of the mutant receptor hGRαT556I into the nucleus (50 min) **(Figure 2B)**, compared with the wild-type receptor (12 min) (**Figure 2A**).

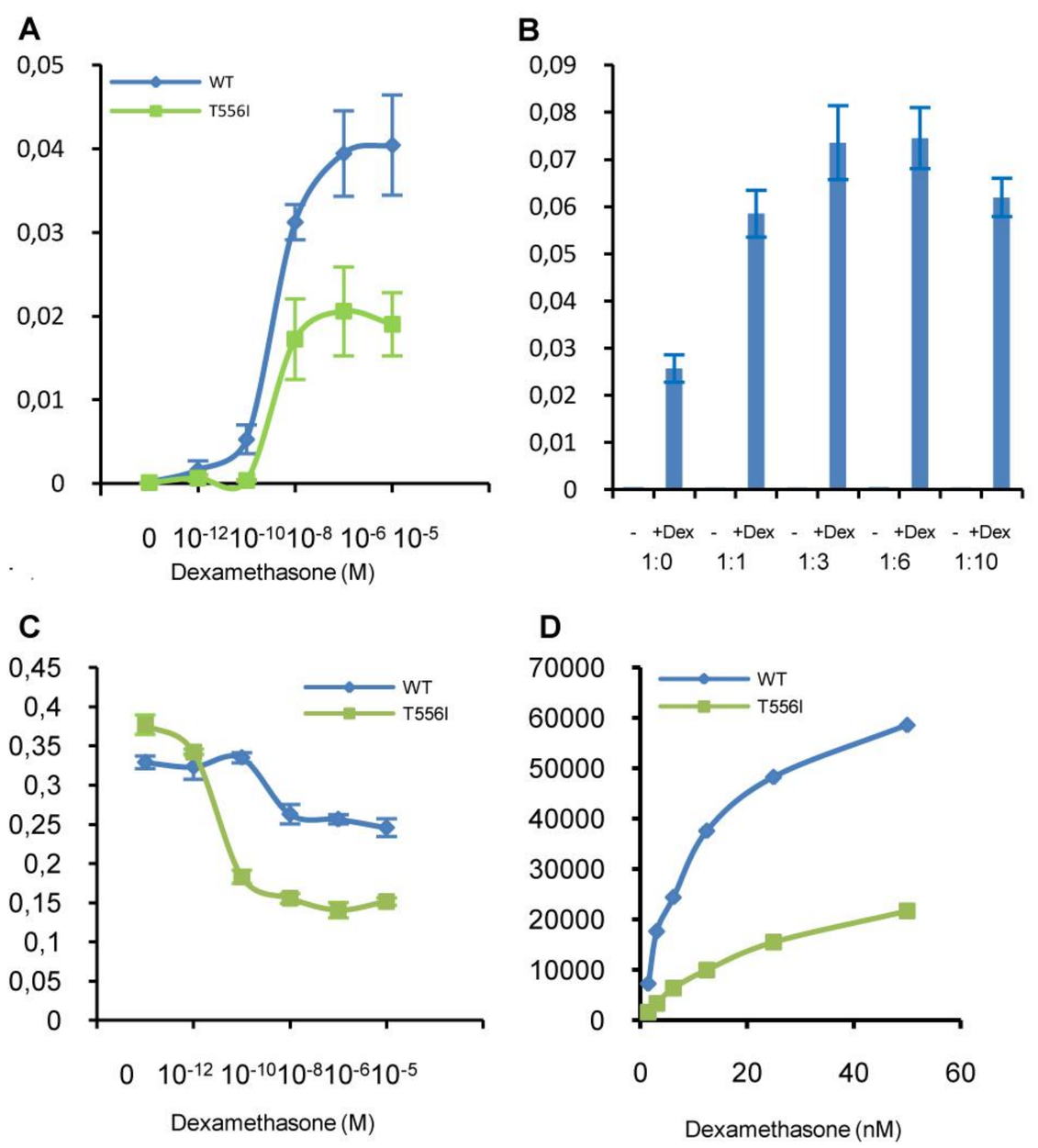

Figure 1: (A) Transcriptional activity of the mutant receptor hGRαT556I compared with the wild-type hGRα. (B) Transdominance assays. (C) Transrepression assays. (D) Dexamethasone-binding assays showed that the mutant receptor hGRαT556I displayed lower affinity for the ligand.

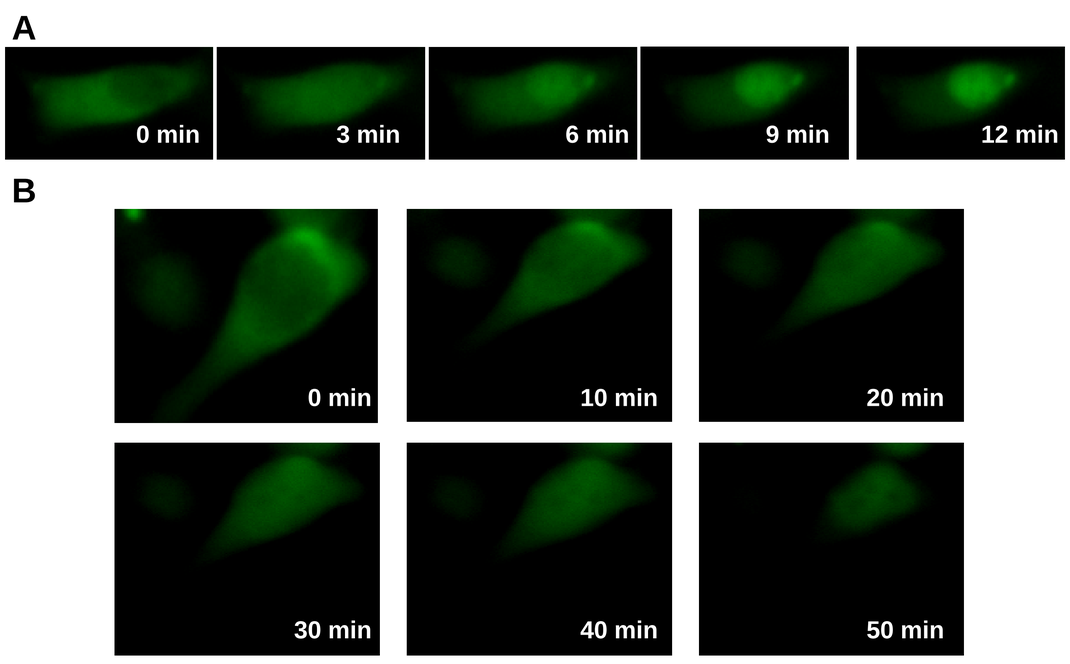

Figure 2: (A) The hGRαWT translocated to the nucleus within 12 min. (B) The mutant receptor hGRαT556I required 50 min to complete nuclear translocation.

The 3D molecular modeling study of the T556I mutation revealed that the -OH moiety of T556 established strong hydrogen bonding interactions with the =O group of P637 backbone. The T556I mutation led to the disruption of the hydrogen bond and significant relocation of the P637 bearing loop, thus affecting mildly the local 3D arrangement of the receptor and hence the electrostatic surface of the region.

CONCLUSIONS

The natural mutant receptor hGRαT556I alters glucocorticoid signal transduction through multiple molecular mechanisms.

REFERENCES

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