TRANSIENT GENERALIZED GLUCOCORTICOID HYPERSENSITIVITY: CLINICAL MANIFESTATIONS, ENDOCRINOLOGIC EVALUATION AND TRANSCRIPTOMIC PROFILE: THE POTENTIAL ROLE OF NF-κB

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INTRODUCTION

Transient Generalized Glucocorticoid Hypersensitivity is a rare disorder characterized by generalized increased tissue sensitivity to glucocorticoids and compensatory hypoactivation of the hypothalamic-pituitary-adrenal (HPA) axis. The condition itself and the molecular mechanisms that underlie its pathophysiology have not been elucidated as yet. Adenovirus 36 has been reported to cause obesity in various animal species.

OBJECTIVE AND HYPOTHESES

To present the clinical manifestations, endocrinologic evaluation and transcriptomic profile in a rare pediatric case of Transient Generalized Glucocorticoid Hypersensitivity.

PATIENT AND METHODS

Case report

A 9-year old girl presented with an 8-month history of clinical manifestations suggestive of Cushing syndrome, including central obesity, moon facies, buffalo hump, purple striae, hypertrichosis and decreased growth velocity (**Figures 1B-D**). Endocrinologic evaluation revealed undetectable 08:00h ACTH (< 1 pg/mL) and cortisol (0.025 μg/dL) concentrations that remained similarly decreased throughout a 24h period of study and did not respond to ovine CRH stimulation (1 μg/kg). The disease gradually resolved spontaneously over the ensuing 3 months.

DNA extraction and sequencing analysis

DNA was extracted from peripheral blood mononuclear cells (PBMCs) and the entire coding region of the *hGR* gene was amplified and sequenced.

Thymidine incorporation assays

PBMCs from the patient during illness were isolated using Ficoll-Paque Plus. Thymidine incorporation assays were performed as previously described (**1**).

Total RNA isolation

Total RNA was purified from PBMCs obtained from the patient during illness and after recovery using Trizol® Reagent and treated with DNase I.

RNA sequencing

RNA-Seq was performed using Illumina HiSeq2000, which generated 33.8 and 32.3 million 36bp single end reads for the two samples.

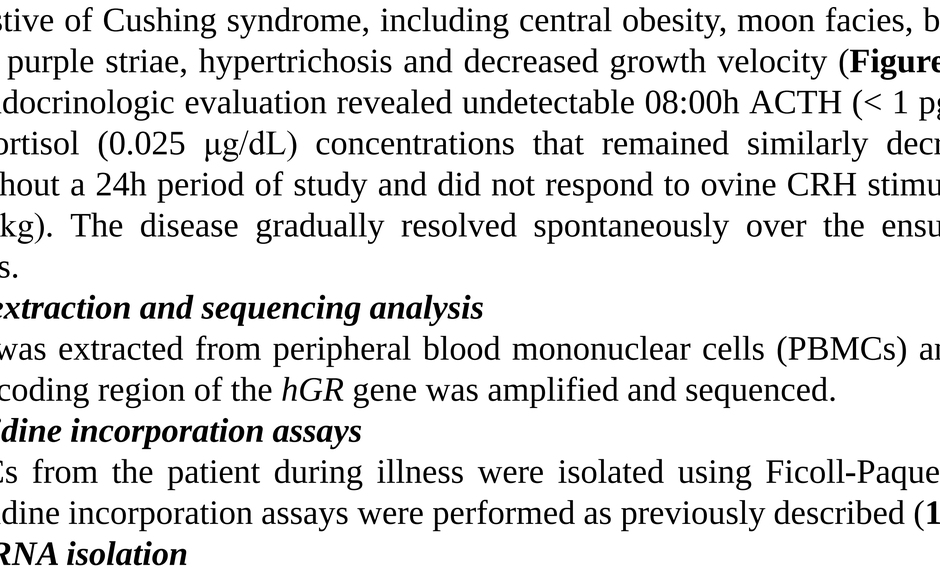

Figure 1: The patient before (A) and after (B, C, D) the onset of clinical manifestations of Transient Generalized Glucocorticoid Hypersensitivity.

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RESULTS

No mutations or polymorphisms were found in the *hGR* gene of the patient. The thymidine incorporation assays performed on fresh PBMCs revealed increased sensitivity to dexamethasone-induced suppression of phytohemagglutinin-stimulated thymidine incorporation in the patient compared with a matched control subject (**Figure 2**).

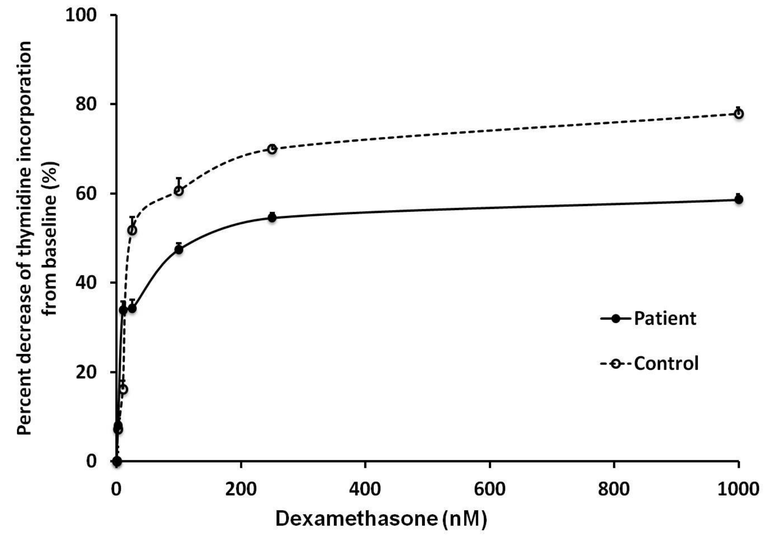

Figure 2: Thymidine incorporation assays.

Transcriptomic (RNA sequencing) analysis in white blood cells in the disease and post resolution phases identified 903 differentially expressed genes. Of these, 106 genes were up-regulated and 797 were down-regulated in the disease compared with the resolution phase. Bioinformatic analysis on the differentially expressed gene networks revealed Nuclear Factor-κB as the predominant transcription factor influencing the expression of the majority of these differentially expressed genes, suggesting an active inflammatory reaction. RNA-sequencing showed no enrichment of adenovirus 36 sequence in our patient.

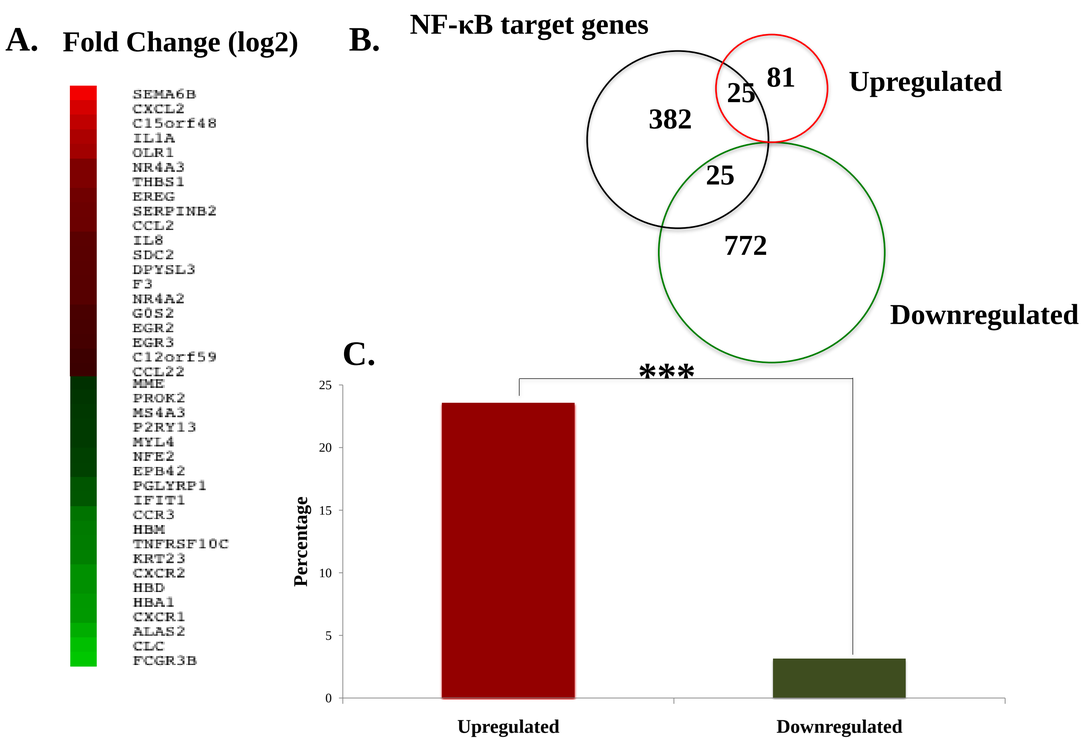

Figure 3: (A) Top 20 up- and down-regulated genes in the disease compared to resolution phase. (B) Venn diagram comparing the NF-κB target genes in the up-regulated and down-regulated population. (C) Bar graph of enrichment of the NF-κB target genes in the up-regulated and down-regulated gene population.

CONCLUSIONS

Our findings indicate that a transient post-receptor defect or a virus-encoded molecule may have interfered with glucocorticoid signal transduction leading to Transient Generalized Glucocorticoid Hypersensitivity in our patient. The changes in the transcriptome in the active phase of the disease point toward a transient exogenous insult, probably an infectious agent.

REFERENCE

1. Charmandari *et al. J Clin Endocrinol Metab.* 2005; 90(6): 3696-3705 .