Understanding immunogenetics of autoimmune Graves' disease (GD) in patients of West Bengal, a cross-sectional study

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Abstract

Background

Graves' disease (GD), the most prevalent autoimmune thyroid disorder, is characterized by thyroid-specific autoantibody production, hyperthyroidism, lymphocytic infiltration, and systemic immune dysregulation. Although both genetic and environmental factors contribute to disease development, the molecular mechanisms underlying GD pathogenesis remain incompletely understood. Emerging evidence suggests that vitamin D receptor (VDR) polymorphisms, HLA class II susceptibility alleles, and endoplasmic reticulum (ER) stress-mediated unfolded protein response (UPR) signaling play critical roles in autoimmune inflammation. The present study investigated the association of VDR polymorphisms (ApaI-rs7975232 and BsmI-rs1544410), HLA class II alleles (HLA-DQB2 and HLA-DRB3), immune dysregulation, and ER stress pathways in patients with Graves' disease from West Bengal, India.

Methods

A cross-sectional study was conducted involving 70 clinically diagnosed GD patients and age- and sex-matched healthy controls. Peripheral blood (5 mL) was collected in EDTA and clot vials for the isolation of peripheral blood mononuclear cells (PBMCs), genomic DNA, RNA, protein extracts, and serum. Serum samples were analyzed for thyroid function parameters (T3, FT4, and TSH), 25-hydroxy vitamin D [25(OH)D], thyroid autoantibodies (TSHR-Ab, anti-TPO Ab, and anti-Tg Ab), and inflammatory cytokines including TNF-α, IL-2, IL-4, IL-6, and IL-17. Most biochemical parameters were assessed using chemiluminescence immunoassay (CLIA)-based commercial kits on the MAGLUMI 4000 semi-automated analyzer, while cytokine levels were quantified by sandwich ELISA. VDR polymorphisms (ApaI-rs7975232 and BsmI-rs1544410) were analyzed using PCR-RFLP. Gene and protein expression analyses of ER stress and immune regulatory markers were performed using qRT-PCR and immunoblotting techniques.

Results

GD patients exhibited marked immune-inflammatory dysregulation characterized by elevated serum thyroid hormone levels, increased titres of thyroid-specific autoantibodies, enhanced expression of pro-inflammatory cytokines, and reduced expression of immune tolerance-associated markers. The VDR *ApaI* (rs7975232) and *BsmI* (rs1544410) polymorphic variants were detected in 65.71% and 64.28% of GD patients, respectively, and were significantly associated with heightened inflammatory signaling, activation of ER stress and unfolded protein response (UPR) pathways, and downregulation of regulatory immune markers such as FoxP3 and CTLA-4. Although circulating 25(OH)D levels did not differ significantly between patients and controls, individuals carrying VDR mutant variants demonstrated greater immune activation and disease severity, suggesting impaired VDR-mediated immunomodulatory signaling rather than absolute vitamin D deficiency. Additionally, HLA-DQB2 and HLA-DRB3 alleles showed strong associations with genetic susceptibility to GD. Elevated expression of ER stress-related molecular markers further supported the role of ER stress and UPR activation in sustaining chronic inflammation, immune dysregulation, and thyroid autoimmunity.

Conclusion

The present study demonstrates that VDR polymorphisms, particularly the *ApaI* and *BsmI* variants, contribute to Graves' disease pathogenesis by promoting inflammatory immune responses, impairing immune tolerance mechanisms, and enhancing ER stress-mediated immune activation. While HLA class II alleles primarily influence genetic susceptibility to GD, VDR variants appear to modulate disease severity and inflammatory burden. Collectively, the

interplay between genetic susceptibility, dysregulated immune responses, and ER stress signaling provides important mechanistic insights into GD immunopathogenesis and highlights potential molecular biomarkers and therapeutic targets for precision-based management of Graves' disease.