Puberty-Driven Gene Expression Shifts in Autism

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Introduction

Puberty is a critical developmental phase characterized by the reactivation of the hypothalamic-pituitary-gonadal (HPG) axis, leading to the release of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). This process triggers the maturation of the gonads, resulting in increased levels of sex steroids such as testosterone and estradiol (E2) [1]. These hormonal changes significantly influence both central neural circuitry and peripheral immune function.

Emerging evidence indicates that pubertal sex steroids, particularly estrogens and androgens, orchestrate immune system maturation. Estrogen enhances B cell proliferation and antibody production while downregulating Th1/Th17-mediated immune responses. In contrast, androgens like testosterone generally suppress pro-inflammatory pathways [2]. Puberty also augments dendritic cell activation and T helper cell polarization, contributing to heightened autoimmune susceptibility during adolescence [3].

At the molecular level, puberty involves extensive transcriptional and epigenetic remodeling within the immune compartment. Longitudinal studies reveal significant shifts in peripheral leukocyte gene expression profiles across pubertal transitions, with a notable switch from innate to adaptive immune signatures in post-menarcheal females [2, 4]. These transitions coincide with DNA methylation changes in immune-related genes, highlighting the coordination between steroid hormone signaling and gene expression [5].

ASD is increasingly characterized by dysregulated immune signaling and altered hormonal sensitivity. However, the intersection of puberty-related immune-endocrine transitions and ASD pathophysiology remains underexplored. Understanding whether puberty amplifies divergent transcriptional responses in ASD could reveal crucial developmental mechanisms that modulate disease trajectories.

To investigate this, we analyzed whole-blood RNA-sequencing data from the GSE212645 dataset, which includes ASD children and unaffected siblings at pre- and post-pubertal stages. Our goal was to determine whether puberty—and the accompanying immune and hormonal remodeling—elicits distinctive transcriptomic responses in ASD, particularly in pathways related to immunity and steroid hormone regulation.

Objective

The primary objective of this study is to investigate how puberty modulates gene expression in individuals with Autism Spectrum Disorder (ASD) compared to their unaffected siblings. By analyzing whole-blood RNA-seq data, we aim to identify differentially expressed genes (DEGs) and biological pathways influenced by the interaction between genotype (ASD vs. sibling) and pubertal status. Particular focus is placed on immune and hormonal signaling pathways, known to undergo critical changes during puberty and implicated in ASD pathophysiology.

Research Question

How does pubertal development alter gene expression profiles in individuals with ASD compared to unaffected siblings, and what biological pathways—particularly those related to immune and hormonal function—are differentially regulated during this transition?

Materials and Methods

The analysis was conducted using R programming language and various bioinformatics packages. The primary script used for the RNA sequencing analysis is available in the repository titled RNA-seq analysis, hosted on GitHub [13]. This repository contains the complete workflow, including data processing, differential expression analysis, and functional enrichment analysis. The specific analysis script can be accessed at GitHub Repository.

1. Dataset Acquisition

Publicly available RNA-sequencing data was obtained from the Gene Expression Omnibus (GEO) under accession number GSE212645 [6]. This dataset comprises whole blood transcriptomes from individuals diagnosed with ASD and their unaffected siblings. Participants were stratified into pre-pubertal and post-pubertal stages based on Tanner staging and/or age classification as reported in the original study.

2. Sample Grouping and Metadata Curation

Subjects were grouped based on:

- **Genotype**: ASD vs. unaffected siblings
- **Pubertal Stage**: Pre-puberty vs. Post-puberty

Sample metadata were curated to ensure correct mapping of individuals across these variables. The experimental design formed a 2×2 factorial structure:

- ASD_Pre
- ASD_Post
- Sibling_Pre
- Sibling_Post

Sex, batch, and age were recorded and examined for potential confounding effects.

3. RNA-Seq Data Processing

Raw count matrices were obtained from the GSE212645 dataset.

3.1 Quality Control

Initial quality control was performed using:

- Principal Component Analysis (PCA) on normalized counts
- Outlier detection and potential batch effects were assessed.

3.2 Gene Filtering

Lowly expressed genes (counts < 10 in more than 80% of samples) were filtered out to improve statistical power and reduce noise.

4. Differential Expression Analysis

Differential gene expression was conducted using DESeq2 (v1.38.3) in R.

4.1 Design Formula

A multifactorial model was applied:

~ Genotype + Puberty + Genotype:Puberty

This design allows for:

- Main effects of ASD (Genotype)
- Main effects of Puberty
- Interaction term capturing differential pubertal effects in ASD vs. siblings

4.2 Contrast Testing

Key contrasts extracted:

- ASD vs. Sibling (overall)
- Pre vs. Post Puberty (overall)
- Interaction: (ASD_Post - ASD_Pre) vs. (Sibling_Post - Sibling_Pre)

4.3 DEG Selection

Significant DEGs were identified using:

- Adjusted p-value (padj) < 0.05
- Absolute log2 fold change > 1 (|log2FC| > 1)

Multiple testing correction was performed using the Benjamini-Hochberg method.

5. Functional Enrichment Analysis

To characterize the biological significance of DEGs, functional enrichment was performed using clusterProfiler (v4.6.0) in R.

5.1 Gene Ontology (GO)

Enrichment analysis was conducted for:

- Biological Process (BP)

Top GO terms were visualized using dot plots and enrichment maps.

5.2 KEGG Pathway Analysis

KEGG pathways enriched among upregulated and downregulated DEGs were identified using enrichKEGG(). KEGG IDs were mapped to gene symbols using the **org.Hs.eg.db** database.

5.3 Immune and Hormonal Pathway Annotation

Special attention was given to pathways related to:

- Immune activation and defense response
- Steroid hormone biosynthesis and signaling

Manual curation and annotation highlighted the immunological and hormonal relevance of key findings.

6. Visualization and Interpretation

- **PCA Plots**: To assess group separation based on gene expression.
- **Volcano Plots**: Visual representation of DEGs with significance and fold change.
- **Heatmaps**: Top 50 DEGs for each contrast visualized across samples.
- **Pathway Dot Plots**: Functional categories enriched in interaction-specific genes.

Results

Differential Gene Expression Analysis

The differential expression analysis revealed significant shifts in gene expression profiles associated with pubertal development in individuals with Autism Spectrum Disorder (ASD) compared to their unaffected siblings. The volcano plots (Figures 1 and 2) illustrate the distribution of differentially expressed genes (DEGs) across the four experimental groups: ASD_Pre, ASD_Post, Sibling_Pre, and Sibling_Post.

In Figure 1, the volcano plot for ASD children indicates numerous significant genes (red dots) that were upregulated post-puberty, with an adjusted p-value (padj) < 0.05 and absolute log2 fold change > 1. These genes are predominantly associated with immune activation and hormonal signaling pathways, suggesting a robust transcriptional response to puberty in individuals with ASD. Contrastingly, Figure 2 displays the volcano plot for unaffected siblings, where minimal significant changes in gene expression were observed during puberty, underscoring the unique transcriptional dynamics present in the ASD group.

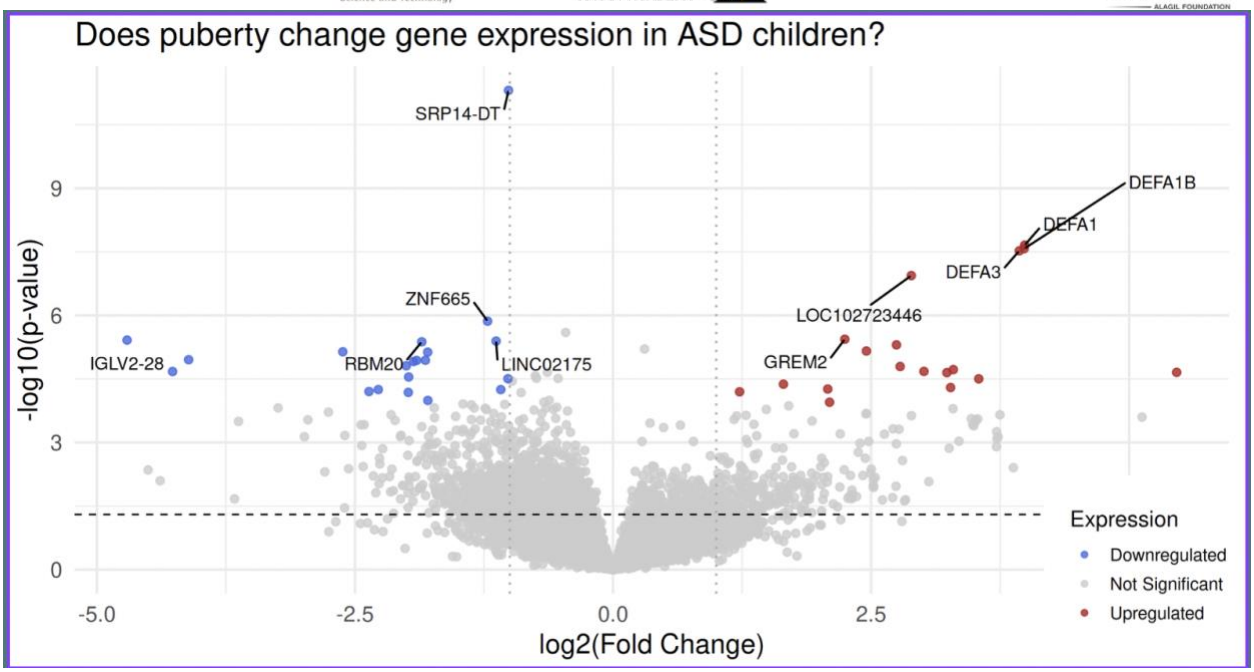


Figure 1: ASD Children: Significant gene expression changes were observed during puberty.

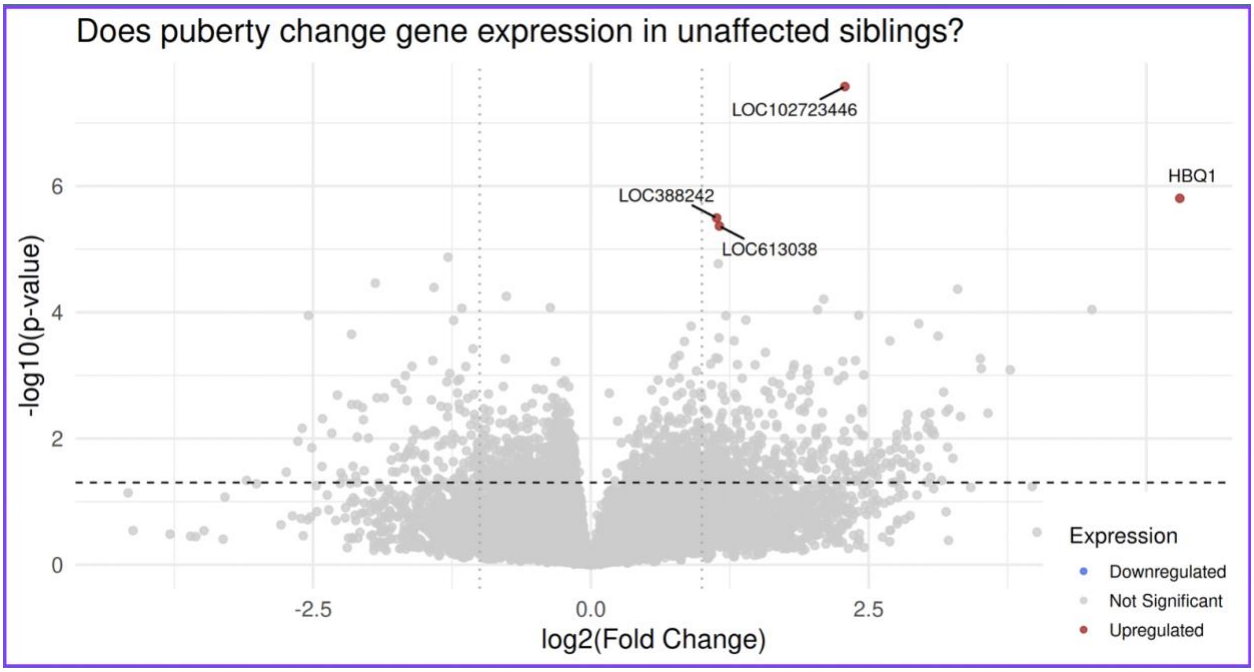


Figure 2: Unaffected Siblings: Minimal significant changes in gene expression were observed during puberty.

Z-Score Expression Trends

The Z-score plot (Figure 3) further elucidates the differential expression patterns between the groups. The plot shows that ASD children (ASD_yes) exhibited more pronounced shifts in both upregulated

(teal) and downregulated (red) gene expression compared to their unaffected siblings (Sibling_no). Notably, the ASD group demonstrated a more substantial increase in upregulated genes post-puberty, which may indicate heightened immune and hormonal responses during this developmental transition.

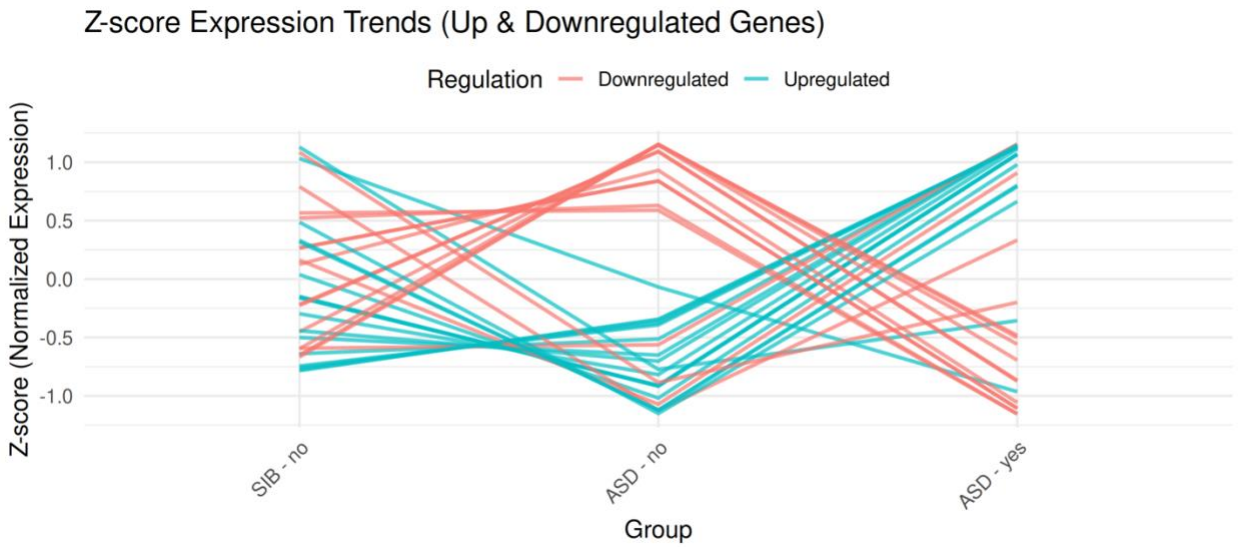


Figure 3: Z-score plot indicates stronger shifts in upregulated (teal) and downregulated (red) genes in the ASD-yes group (treated post-puberty).

Functional Enrichment Analysis

Functional enrichment analysis of the identified DEGs revealed significant biological pathways related to immune activation and steroid hormone signaling. The enriched Gene Ontology (GO) terms and KEGG pathways align with the known roles of sex steroids in modulating immune responses and suggest that puberty amplifies immune dysregulation in ASD.

Discussion

The findings of this study highlight the critical role of puberty in modulating gene expression in individuals with ASD, particularly in immune and hormonal pathways. The significant differences observed between ASD children and their unaffected siblings during puberty suggest that the hormonal changes associated with this developmental phase may exacerbate underlying immunological vulnerabilities in ASD [7].

The extensive transcriptional changes seen in the ASD group, as evidenced by the increased number of DEGs and their functional relevance, support the hypothesis that puberty acts as a potent modifier of disease expression in ASD. The upregulation of immune-related genes aligns with previous literature that documents heightened autoimmune susceptibility during adolescence [8], thereby contributing to our understanding of the interplay between hormonal changes and immune dysregulation in ASD.

Moreover, the minimal changes observed in unaffected siblings during puberty indicate a potential protective mechanism or a more stable gene expression profile in response to hormonal fluctuations. This contrast emphasizes the need for further investigation into the mechanisms underlying these transcriptional responses and their implications for treatment strategies aimed at mitigating the effects of puberty on individuals with ASD [9].

In conclusion, our findings underscore the importance of considering puberty as a significant factor in the gene expression landscape of ASD. Future studies should explore the longitudinal effects of puberty on immune and hormonal regulation in ASD, as well as the potential for targeted interventions during this critical developmental period.

Conclusion

This study elucidates the significant impact of puberty on gene expression in individuals with Autism Spectrum Disorder (ASD), particularly concerning immune and hormonal pathways. Our analysis revealed that puberty induces substantial transcriptional changes in ASD children compared to their unaffected siblings, suggesting that hormonal fluctuations during this developmental phase may exacerbate underlying immunological vulnerabilities. The identification of numerous differentially expressed genes (DEGs) associated with immune activation and hormonal signaling reinforces the notion that puberty serves as a critical inflection point in the expression of ASD-related phenotypes.

These findings contribute to our understanding of the intricate interplay between hormonal changes and immune dysregulation in ASD. The minimal gene expression changes observed in unaffected siblings highlight a potential protective mechanism, emphasizing the need for further research into the biological underpinnings of these differences. Overall, our results underscore the importance of considering puberty as a vital factor influencing the trajectory of ASD.

Recommendations

1. **Clinical Monitoring**: Given the significant transcriptomic changes observed during puberty in individuals with ASD, clinical management should incorporate pubertal status when evaluating symptoms, treatment responses, and behavioral changes.
2. **Hormonal and Immune Profiling**: Comprehensive assessments of hormone levels (e.g., estradiol, testosterone, cortisol) and immune biomarkers in ASD children during puberty may aid in stratifying patients and personalizing interventions.
3. **Integrative Multi-Omics Approaches**: Future studies should combine transcriptomics with epigenomics, proteomics, and metabolomics to gain deeper insights into the mechanisms underlying puberty-related changes in ASD.

Future Work

1. **Longitudinal Studies**: Conducting long-term studies that track individuals across different stages of puberty is essential to confirm the causal relationships and temporal dynamics of hormone-immune interactions in ASD.
2. **Cell-Type-Specific Expression Analysis**: Employing single-cell RNA sequencing or cell deconvolution methods will help elucidate transcriptomic changes within specific immune cell types that may be masked in whole-blood analyses.
3. **Functional Validation**: Future research should focus on validating the roles of identified genes and pathways through in vitro or in vivo experiments, utilizing techniques such as CRISPR or hormone stimulation assays.
4. **Therapeutic Implications**: Investigating how modulation of hormone levels or immune pathways influences ASD phenotypes during puberty could lead to novel, targeted interventions during this critical developmental window.

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