Maternal Insulin Therapy and Its Impact on Immune Regulation in Infants of Mothers with Kidney Disease and Gestational Diabetes

A comprehensive investigation into the immunological effects of maternal insulin therapy during gestational diabetes mellitus and its implications for neonatal immune development and kidney disease risk.

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COI Disclosure

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Introduction:

- Gestational diabetes mellitus (GDM) is characterized by carbohydrate intolerance during pregnancy, typically beginning around the 20th week. This condition poses significant risks to both the mother and the fetus.
- GDM is a metabolic disorder that can influence fetal immune development, particularly in infants born to mothers with pre-existing kidney disease. Given the intricate relationship between metabolic dysfunction, inflammation, and renal health, this study explores how maternal insulin therapy modulates immune responses in utero.
- Regulatory T cells (Tregs), a subset of CD4+ T cells, are crucial for maintaining immune tolerance by suppressing the activation of various immune cells. The transcription factor FOXP3 is essential for Treg development and function, with Tregs secreting anti-inflammatory cytokines like IL-10 and transforming growth factor (TGF)-β to mediate suppression.
- Understanding the effects of insulin therapy on neonatal immune regulation is essential, as GDM is a common pregnancy complication affecting fetal immune system development.
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Objectives:

- Maternal insulin therapy during gestational diabetes mellitus (GDM) induces regulatory immune mechanisms in utero, potentially modulating foetal immune development and subsequent disease susceptibility.

- Specifically, we investigated the effect of insulin on CD4+CD25+FOXP3+ regulatory T cells development, proinflammatory cytokine production, chemokines, autoantibodies and immune gene expression in the cord blood of infants born to mothers with kidney disease and GDM.

Methodological Approach

Cord blood mononuclear cells (CBMCs) were collected from 124 infants born to mothers with kidney disease and GDM and 110 infants born to unaffected mothers.

01

Cell Isolation

Cord blood mononuclear cells were isolated using density gradient centrifugation immediately following delivery to preserve cell viability and functional integrity.

02

Flow Cytometry Analysis

CD4+CD25+FOXP3+ regulatory T cells were quantified both ex vivo and following in vitro insulin stimulation to assess baseline and responsive immune profiles.

03

Gene Expression Profiling

RT-PCR analysis examined mRNA expression of FOXP3, NFATc2, STIM1, IL-10, IFN-γ, TNF-α, and TGF-β to characterize immune regulatory mechanisms.

04

Autoantibody Assessment

Anti-GAD65 autoantibody levels were measured and correlated with immune markers to evaluate autoimmune risk stratification.

Analysis

- ❖Flow cytometry for **CD4+CD25+FOXP3+ cells**
- ❖In vitro stimulation with **human insulin**
- ❖Measurement of cytokines, chemokines, and autoantibodies

Results

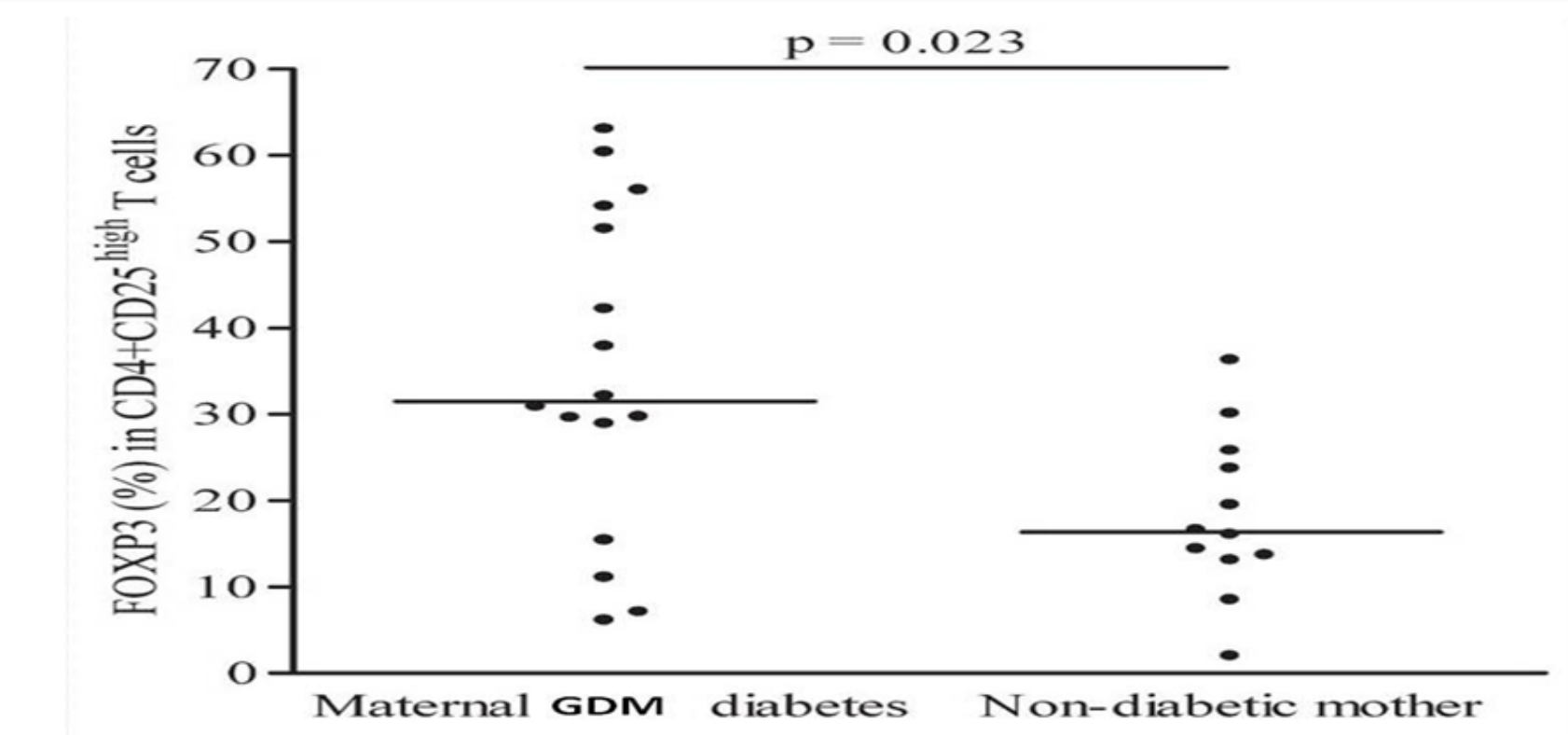

Fig 1. Flow cytometric analysis of the percentage of FOXP3+ cells among CD4+CD25high regulatory T cells in cord blood.

Cord blood mononuclear cells were stained with CD4, CD25, FOXP3, and isotype control. The expression of FOXP3 in CD4+CD25high regulatory T cells is presented in infants with maternal diabetes with kidney disease and infants of non-diabetic mothers.

Values represent the proportion (%) of FOXP3-positive cells. Horizontal lines represent median values. p Value derived from the Mann–Whitney U test is shown.

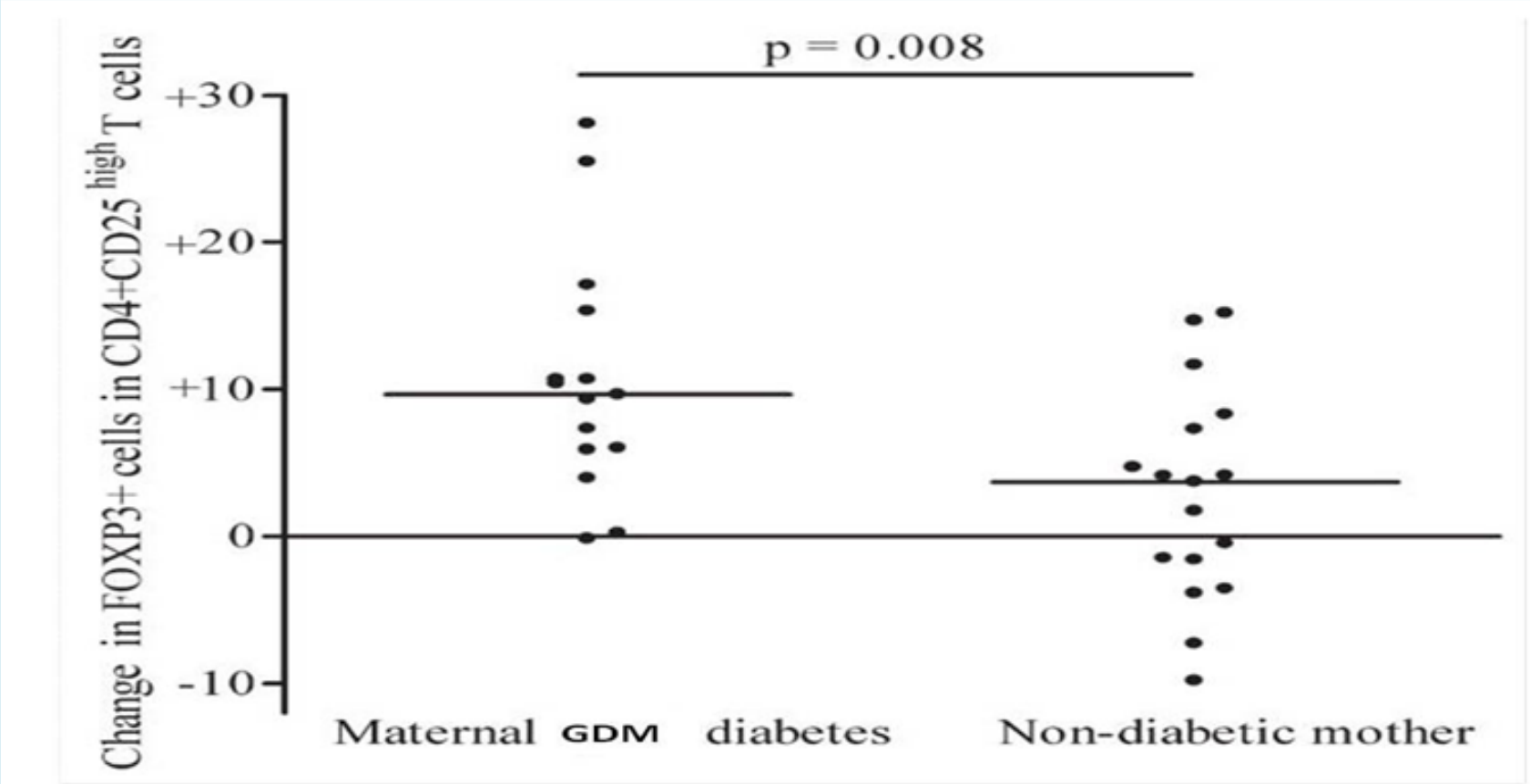

Fig 2. The increase in the proportion of FOXP3-positive cells in CD4+CD25high regulatory T cells in infants with maternal diabetes with kidney disease and infants of non-diabetic mothers after 72 h stimulation with human insulin.

Cord blood derived mononuclear cells were stained with CD4, CD25, FOXP3, and isotype control and analyzed with flow cytometry. Horizontal lines represent median values.

p Value comparing the in vitro insulin-induced change in the numbers of FOXP3 expressing CD4+CD25high cells between the study groups (Mann–Whitney U test) is shown.

Enhanced Regulatory T Cell Populations in GDM Offspring

Cord blood analysis revealed a significantly elevated proportion of FOXP3+ cells within the CD4+CD25(high) population in infants born to mothers with kidney disease and GDM compared to those from unaffected mothers.

This finding suggests that maternal insulin therapy during pregnancy may prime the foetal immune system towards a regulatory phenotype, potentially conferring protective effects against autoimmune conditions such as type 1 diabetes.

The increased Treg frequency represents a fundamental shift in the neonatal immune landscape, with implications for long-term immune regulation and disease susceptibility.

Clinical Significance

Elevated FOXP3+ Tregs in GDM offspring may represent an adaptive immune response to maternal metabolic disturbance, with potential protective effects against autoimmune diabetes development.

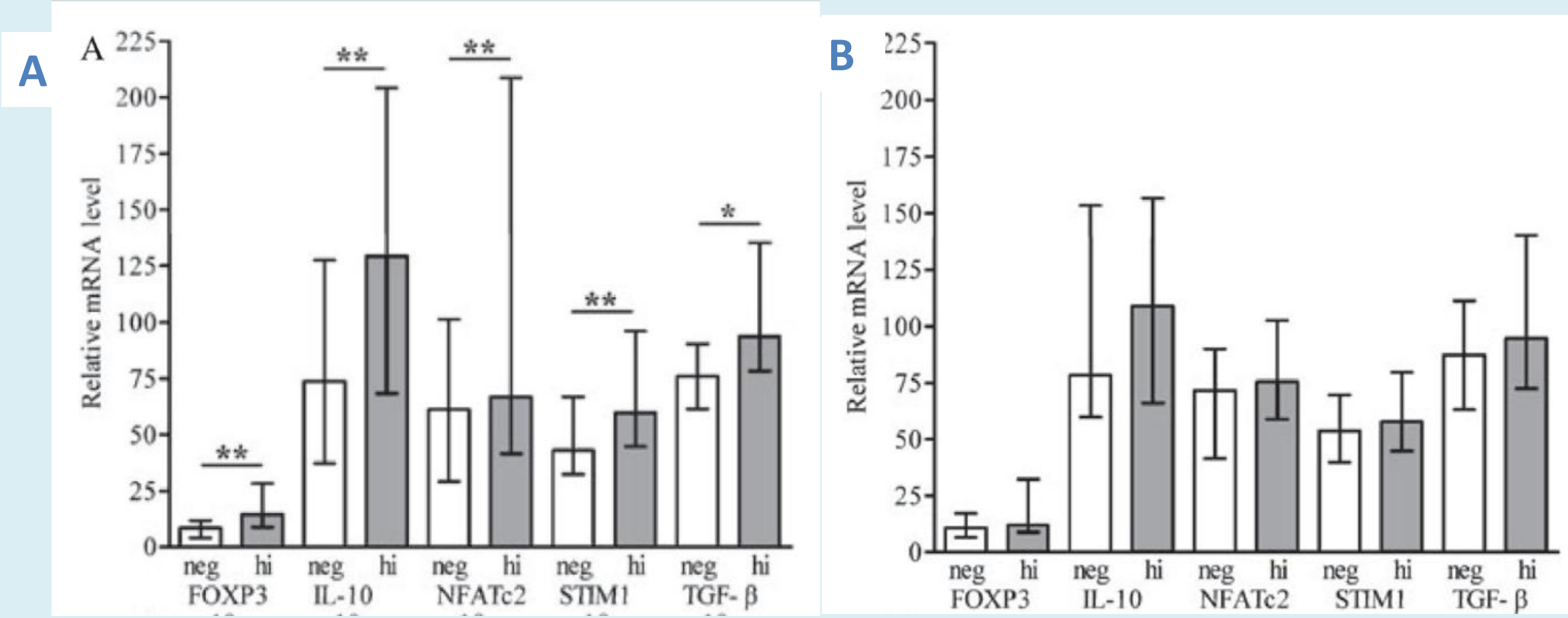

Fig 3. Expression of FOXP3, IL-10, NFATc2, STIM1, and transforming growth factor (TGF)-β specific mRNA in cord blood mononuclear cells in infants of diabetic GDM mothers with kidney disease (A) and of unaffected mothers (B) after 72 h stimulation with human insulin (hi) or medium alone (neg).

In infants with maternal diabetes FOXP3, NFATc2, STIM1, IL-10, and TGF-β-specific mRNA increased significantly in CBMCs in response to insulin ($p < 0.001$) , whereas no such increase was seen in the infants of non-diabetic mothers.

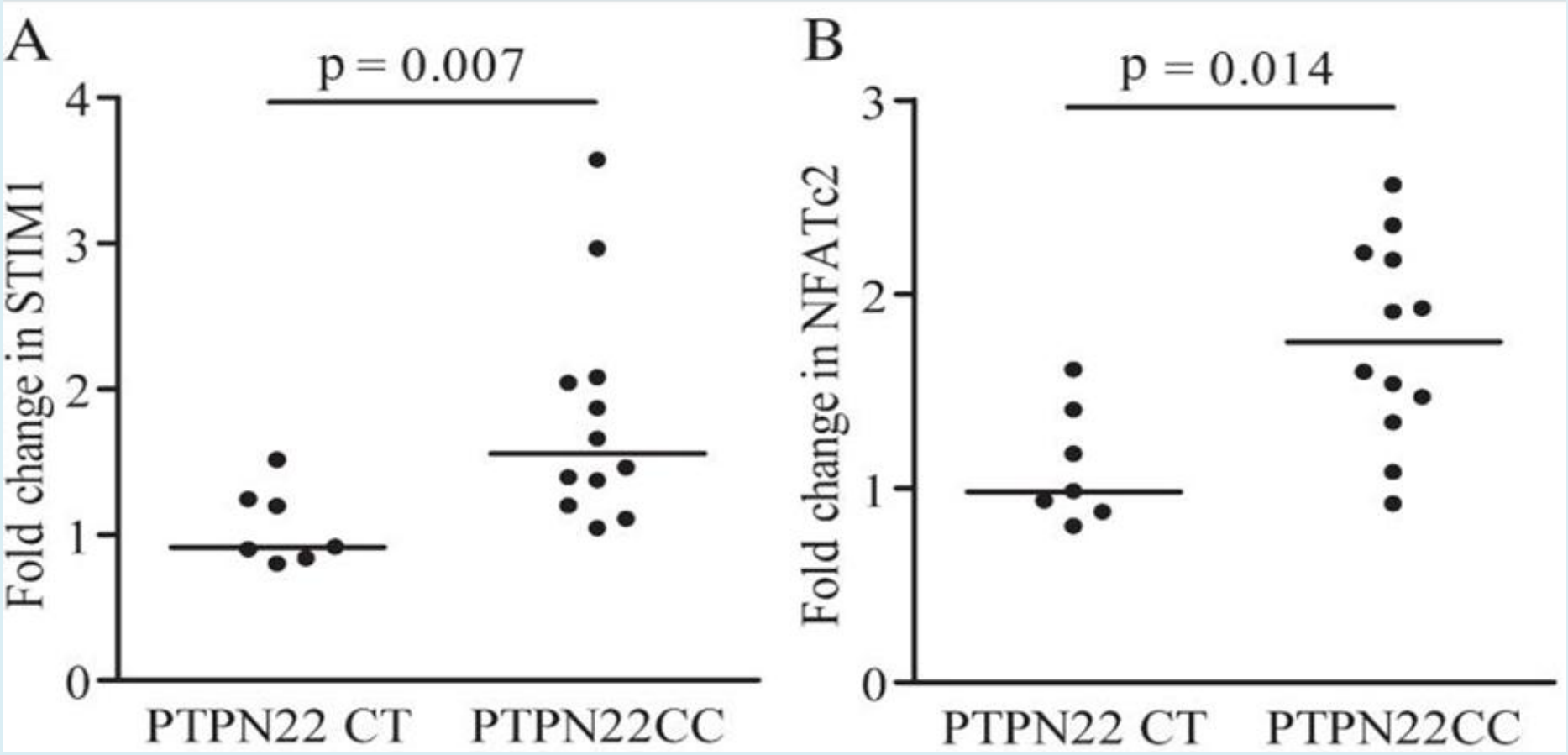

Fig. 4. Upregulation of STIM1 and NFATc2-specific mRNA expressed as fold change in relation to the *PTPN22 genotype in infants of mothers with kidney disease and GDM diabetes.*

Fold change in STIM1 (A) and NFATc2 (B) was calculated by dividing the relative mRNA level of insulin-stimulated (72 h) cord blood mononuclear cells (CBMCs) by the relative mRNA level of non-stimulated CBMCs. Horizontal lines represent median values. p Values comparing the groups (Mann–Whitney U test) are shown.

Genetic Polymorphism and Immune Response Variation

PTPN22 Allele Carriers

Infants carrying the PTPN22 genetic variant demonstrated reduced activation of STIM1 and NFATc2 following insulin stimulation. This polymorphism, associated with autoimmune susceptibility, appears to attenuate insulin-mediated immune regulatory responses.

Autoantibody Correlation

Higher anti-GAD65 autoantibody levels were observed in conjunction with elevated immune cell frequencies, suggesting ongoing immune education processes that may influence long-term autoimmune risk stratification.

Proinflammatory Markers

TNF-α and IL-10 levels were significantly elevated in the study cohort, correlating positively with increased frequency of CD4+CD25+ T cells. These findings indicate a complex immune environment balancing regulatory and inflammatory signals.

Higher Baseline Tregs in High-Risk Infants

Infants of mothers with kidney disease + GDM had:

✓ Higher $FOXP3^{+}$ Treg levels

✓ Higher $CD4^{+}CD25$(high) percentages

Results :

- Infants of mothers with kidney disease and GDM exhibited a significantly higher percentage of FOXP3+ regulatory T cells within the CD4+CD25(high) population compared to those born to obese mothers without GDM.

- **T Cell Regulation**
- Higher **FOXP3+ cells** in CD4+CD25(high) subset in neonates of GDM mothers.
- Increased **FOXP3, NFATc2, STIM1, IL-10, and TGF-β** after insulin stimulation in GDM group.

- **Cytokines & Autoantibodies**
- Elevated **TNF-α, IL-10**, and **CD4+CD25+ T cells** in neonates of GDM mothers.
- Positive correlation between **anti-GAD65 autoantibodies** and immune changes.

- In infants of non-diabetic mothers there was no difference between FOXP3 expression in CD4+CD25high T cells after stimulation with insulin compared with unstimulated CBMCs.

Discussion

- Our findings support the potential for insulin-specific immune interventions to prevent gestational diabetes mellitus (GDM) with kidney disease.
- We observed an increase in circulating CD4+CD25 highFOXP3+ regulatory T cells (Tregs) in infants of mothers with kidney disease and GDM compared to those from unaffected mothers. This indicates that maternal insulin treatment may expand Tregs and enhance pro-inflammatory cytokine production, contributing to a lower diabetes risk in children of diabetic mothers compared to those of diabetic fathers.
- If genetics were the sole factor, the diabetes risk in children would be similar regardless of whether the mother or father is diabetic. However, the reduced risk among children of diabetic mothers suggests protective non-genetic factors at play. Maternal insulin therapy enhances Treg expansion, promoting immune tolerance, while elevated TNF-α and IL-10 levels reflect a dual pro-/anti-inflammatory response.
- Maternal history was a significant risk factor for neonatal allergies, while reduced Treg activity, lower IL-10 levels, and decreased FOXP3 expression (in lipid A and peptidoglycan-stimulated cells) were independent risk factors for allergies in offspring, even after controlling for maternal allergy and environmental exposures.

Conclusions and Future Directions

Key Finding 1

Maternal insulin therapy during kidney disease with gestational diabetes mellitus enhances foetal regulatory T cell populations and function, potentially reducing long-term diabetes risk in offspring through persistent immunological programming.

Key Finding 2

Maternal kidney disease with GDM impair regulatory T cell function in cord blood, constituting an independent risk factor for early childhood allergic disease development, even after controlling for genetic and environmental confounders.

Implications for Clinical Practice

Maternal insulin therapy enhances regulatory T cells and modulates inflammation in infants of mothers with kidney disease and GDM, potentially improving renal resilience and reducing autoimmune risk.

Longitudinal studies following these cohorts into childhood will elucidate the persistence of these immunological signatures and their relationship to clinical disease outcomes, informing precision medicine approaches to perinatal care.

Acknowledgments

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