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ORIGINAL ARTICLE

V-set immunoregulatory receptor gene mutation and neonatal combined immunodeficiency in a consanguineous family

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ABSTRACT

Objective: To explore the potential association between a *VSIR* gene mutation and primary combined immunodeficiency (CID) in a patient from a consanguineous family.

Methods: This family-based genetic study was conducted after obtaining ethical approval from the Institutional Review Board of HBS Medical and Dental College, Islamabad (Ref: HBS/IRB/19/25). A 2-month-old female presenting with recurrent infections was clinically evaluated. Laboratory investigations included complete blood count, serum immunoglobulins, and lymphocyte subset analysis by flow cytometry. Whole exome sequencing (WES) was performed to identify potential genetic variants, followed by variant filtering, in-silico pathogenicity prediction, and segregation analysis using Sanger sequencing in available family members.

Results: The patient presented with recurrent infections, marked leukocytosis (WBC: 86.5 × 10^9/L), thrombocytosis, and hypogammaglobulinemia (low IgA, IgM, and IgG). Flow cytometry demonstrated elevated $CD3^+$, $CD4^+$, and $CD8^+$ T-cell counts with reduced NK cells. Whole-exome sequencing identified a rare homozygous missense variant in the *VSIR* gene (*c.593C>T; p.Thr198Met*), encoding VISTA, a negative immune checkpoint regulator. The variant lies in a conserved region, has a very low allele frequency (0.00008122), and was predicted to be deleterious (CADD score: 26.2). Segregation analysis confirmed autosomal recessive inheritance, and no other pathogenic variants were identified in known primary immunodeficiency genes.

Conclusion: This study highlights a potential role of *VSIR* mutations in immune dysregulation and combined immunodeficiency, particularly in consanguineous populations. The identified variant may contribute to abnormal T-cell regulation and hypogammaglobulinemia. Further functional studies are required to establish causality and clarify the role of VISTA in immune homeostasis.

Keywsords: Primary Immunodeficiency (MeSH); VISTA (MeSH); *VSIR* (MeSH); T-cell regulation (Non-MeSH); Hypogammaglobinemia (Non-MeSH); Mutation, Missense (MeSH); Homozygous missense variant (Non-MeSH); Consanguinity (MeSH); Immune Dysregulation (MeSH).

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INTRODUCTION

Primary immunodeficiency disorders (PIDs) comprise a heterogeneous group of inherited conditions characterized by increased susceptibility to infections, along with a predisposition to autoimmunity and malignancy resulting from defects in immune function.[1] PIDs may involve abnormalities in either the adaptive or innate immune system. Adaptive immunity is primarily mediated by T and B lymphocytes, whereas innate immunity constitutes the first line of defense and involves myeloid cells, natural killer (NK) cells, and other innate immune components.[2]

T cells play a central role in cell-mediated immunity. Defects in T-cell development or function result in T-cell immunodeficiency, whereas abnormalities in B-cell maturation led to antibody-deficiency disorders. Given the close functional interplay between T and B lymphocytes, T-cell defects may also give rise to combined immunodeficiency disorders (CIDs).[3,4] The clinical manifestations of these conditions vary according to the underlying genetic defect.

Immune dysregulation is closely associated with the development of autoimmune diseases. In such disorders, lymphocyte dysfunction may lead to loss of immune tolerance, resulting in exaggerated autoreactivity and subsequent tissue damage.[1] For example, defects in immune checkpoint pathways have been implicated in pathological processes such as renal fibrosis.[5] Several immune checkpoint molecules and receptors are critical for the regulation of T-cell responses, and their dysfunction may contribute to both immunodeficiency and autoimmunity. One such molecule is the V-domain Ig suppressor of T-cell activation (VISTA), encoded by the *VSIR* gene (OMIM: 615608), located on chromosome 10q22.1 within an intronic region of the *CDH23* gene.[6] VISTA is a constitutively expressed immune checkpoint regulator present on a wide range of hematopoietic cells, where it maintains peripheral T-cell quiescence and modulates immune activation.[7,8] In addition, VISTA has been shown to regulate the activity of natural killer cells, dendritic cells, and macrophages, underscoring its broader

role in immune homeostasis.[9]

While VISTA has been investigated extensively in association with its roles in cancer immunity, its role in inherited immunodeficiency syndromes remains largely unexplored.[10] However, studies suggest that abnormalities in the *VSIR* gene have been linked to an inhibition of T cell proliferation and reduced cytokine production,[11] hinting at a possible link between *VSIR* mutations and primary T-cell immunodeficiency.

This study was planned to identify a novel variant in the *VSIR* gene through whole-exome sequencing in a family-based genetic investigation in a patient with a novel variant in the *VSIR* gene identified through whole-exome sequencing. The affected individual demonstrated clinical and immunophenotypic features consistent with T-cell dysfunction. The findings would play a potential role of *VSIR* in human T-cell immunobiology and expand the spectrum of candidate genes implicated in primary immunodeficiency disorders.

METHODS

Ethical approval for this study was obtained from the Institutional Review Board of HBS Medical and Dental College, Islamabad, Pakistan (Reference #: HBS/IRB/19/25; dated: March 26, 2025). Written informed consent was obtained from the parents and participating healthy siblings for access to clinical and family history, collection of blood samples, and publication of the study findings.

This study was designed as a family-based genetic investigation to identify a potential disease-causing variant. Clinical evaluation and patient recruitment were conducted at Military Hospital (MH) Rawalpindi, Pakistan, while laboratory investigations, including complete blood count (CBC), serum immunoglobulin levels, and flow cytometry, were performed at the Armed Forces Institute of Pathology (AFIP). DNA extraction and PCR were carried out at HBS Medical College, Islamabad.

A 2-month-old female, born to consanguineous parents following an uneventful full-term delivery, presented with fever, persistent cough, and a single episode of seizure. She remained well until 1.5 months of age, after which symptoms developed. Initial laboratory evaluation revealed marked leukocytosis, prompting referral to Aga Khan University Hospital (AKUH). Family history was significant for adverse obstetric outcomes and early childhood deaths. Based on early-onset infections and consanguinity, an underlying primary immunodeficiency was suspected. A three-generation pedigree was constructed to assess the inheritance pattern.

Immunological assessment was performed using flow cytometry on lysed peripheral whole blood with a viability index of 99%. Samples were incubated with fluorochrome-conjugated monoclonal antibodies targeting CD3, CD4, CD8, CD19, and CD56, and analyzed using a 5-color Cytomics FC500 flow cytometer (Beckman Coulter). Gating was performed on bright CD45-positive lymphocytes, and results were interpreted using age-specific reference ranges.

Peripheral blood samples (3 mL) were collected in K_2-EDTA tubes from the patient, parents, and healthy siblings for hematological and genetic analysis. Genomic DNA was extracted from peripheral blood leukocytes using the Axygen® Genomic DNA Miniprep Kit, according to the manufacturer's protocol. Whole-exome sequencing (WES) was performed using the GRCh38 reference genome, with exome capture followed by sequencing on an Illumina platform at St. Anna Children's Cancer Research Institute and the CeMM Research Center for Molecular Medicine, Vienna, Austria. Sequence reads were aligned using BWA-MEM, and variant calling was performed using GATK HaplotypeCaller in accordance with best-practice guidelines.

Variant analysis followed a standard filtering and prioritization strategy. Common variants reported in population databases were excluded, and analysis focused on rare coding and splice-site variants (minor allele frequency <1%) with potential functional impact, excluding synonymous variants. Candidate variants were evaluated based on the expected inheritance pattern, and segregation analysis was performed in available family members. The potential pathogenicity of prioritized variants was assessed using in-silico prediction tools and functional annotation databases.

The identified disease-causing *VSIR* variant was confirmed by Sanger sequencing in the patient. A three-generation pedigree was constructed to assess the inheritance pattern, which was consistent with autosomal recessive transmission in the context of consanguinity (Figure 1A). Segregation analysis was subsequently performed in both parents and available healthy siblings to evaluate co-segregation of the variant with the disease phenotype, thereby validating the variant and confirming its autosomal recessive inheritance pattern (Figure 1B).

RESULTS

The enrolled patient, a 2-month-old female born to consanguineous parents, presented with recurrent fever (101°F), cough, and a single episode of seizure at the age of 1.5 months. She showed no signs of lymphadenopathy, visceromegaly, skin rash, or ear infection. Her blood cultures showed no growth during the hospitalization period. Her umbilical cord had shed normally on her 8th day of life (ruling out the possibility of a case of leukocyte adhesion deficiency). Her clinical history, early childhood deaths in her family, and recurrent infections raised suspicion for a primary imm-unodeficiency disorder.

The initial laboratory workup included a complete blood count (CBC) and a serum immunoglobulin profile. The evaluation, shown in Table I, revealed an elevated white blood cell (WBC) count of 86.5×10^9/L, with a neutrophil percentage of 64.1% and a lymphocyte percentage of 33.9%. The platelet count was also elevated at 1099×10^9/L. The hemoglobin (Hb) and hematocrit (HCT) levels were normal and within the age-appropriate ranges (12.5 g/dL and 36.9%, respectively). Serum immunoglobulin levels shown in Table I, were significantly reduced, with low levels of IgA (<0.15 g/L), IgM (0.2 g/L), and IgG (2.05 g/L), all of these consistent

Table I: Complete blood count and serum immunoglobulins

Test	Result	Reference Range
Hemoglobin	12.5 g/dL	9.0-13.5 (g/dL)
Hematocrit	36.9%	29%-41%
RBC	3.69 × 10^{12}/L	2.7-4.9 (× 10^{12}/L)
MCV	97.8 fL	77-116\5 (fL)
MCH	32.0 pg	26-34 (pg)
MCHC	32.7 g/dL	29-37 (g/dL)
WBC	86.5 × 10^{9}/L	7.1-14.7 (× 10^{9}/L)
Neutrophils	64.1%	8.9%-68.2%
Lymphocytes	33.9%	37.8%-86.7%
Eosinophils	0.5%	0.0%-4.1%
Monocytes	4.9%	3.8%-15.5%
Basophils	0.2%	0.0%-0.5%
Platelets	1099 × 10^{9}/L	210-500 (x 10^{9}/L)
Serum IgA	<0.15 g/L	0.4-3.5
Serum IgM	0.2 g/L	0.5-3.0
Serum IgG	2.05 g/L	6.5-16.0

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; WBC: White Blood Cell (count); IgA: Immunoglobulin A

Table II: Lymphocyte subset analysis

Test	Result	Reference Range (/μL)
$CD3^+$ Total T-lymphocytes	14,018 /μL	2,800-3,500
$CD4^+$ T-helper lymphocytes	6,780 /μL	1,700-2,400
$CD8^+$ T-regulatory lymphocytes	3,535 /μL	800-1,100
$CD19^+$ Total B-lymphocytes	2,196 /μL	1,000-1,700
$CD56^+$ Natural Killer cells	257.0 /μL	300-800

CD: Cluster of Differentiation

with a hypogammaglobulinemic state. HIV serology was negative.

Lymphocyte immunophenotyping by flow cytometry revealed markedly elevated absolute counts of CD3+ (Total T-lymphocytes), CD4+ (T-helper lymphocytes), and CD8+ (cytotoxic T lymphocytes) cells, with absolute counts of 14018.0, 6780.0, and 3535.0, respectively. All lymphocyte subset counts and immunoglobulin levels were interpreted using age-specific reference ranges. The absolute count of CD19+ (B-lymphocytes) cells was mildly high and CD56+ (Natural Killer) cells were moderately low. The patient maintained a CD4/CD8 ratio within the normal range (Table II).

Whole exome sequencing identified a rare homozygous missense variant in the *VSIR* gene: *c.593C*>T, resulting in the amino acid substitution *p.Thr198Met*. This variant was not previously reported in a homozygous state in the gnomAD database and has an extremely low allele frequency (gnomAD allele frequency = 0.00008122, with no reported homozygotes). Predictive algorithms indicate a potentially deleterious effect (CADD score: 26.2). No other definitive pathogenic variants explaining the phenotype were identified in known primary immunodeficiency genes. Sanger sequencing confirmed the segregation of the identified variant *c.593C*>T in the gene *VSIR* in the patient, parents and available healthy siblings (Figure 1B).

DISCUSSION

The patient's early-onset infections, lymphoproliferative markers (leukocytosis and thrombocytosis), and markedly reduced immunoglobulin levels suggest a significant defect in adaptive immune function. With clinical and laboratory results being consistent with a combined immunodeficiency, whole exome sequencing identified a rare homozygous missense variant in the *VSIR* gene (*c.593C*>T; *p.Thr198Met*). The variant lies in a well-conserved region of the protein and was determined pathogenic by multiple in-silico tools (including a high CADD score of 26.2). It is a strong candidate for a pathogenic variant because it is in the homozygous form, and the patient has a clinical immunodeficiency phenotype, as well as consanguineous parents. Notably, no other documented pathogenic variants in known PID-associated genes were found, which further supports *VSIR* as a possible direct cause of disease in this case. The *VSIR* (OMIM: 615608) gene encodes the V-domain Ig suppressor of T cell activation (VISTA), a negative immune checkpoint molecule predominantly expressed on hem-atopoietic cells.[11] VISTA is important in establishing T cell quiescence, modulating T cell activation thresholds, and preventing excessive inflammation, as suggested by the results in Table III. Recent studies have already documented the pathogenic potential for variants in other checkpoint genes, such as *CTLA4*, *LRBA*, and *PIK3CD*, in patients with combined immunodeficiency.[12,13] VISTA may be just as important in allergy responses, autoimmunity, and transplant rejection, due to its immunomodulatory functions.[11] VISTA's expression on myeloid cells in this regard, and its ability to suppress T cell proliferation are important to help frame its function in imm-unosuppression and regulation.

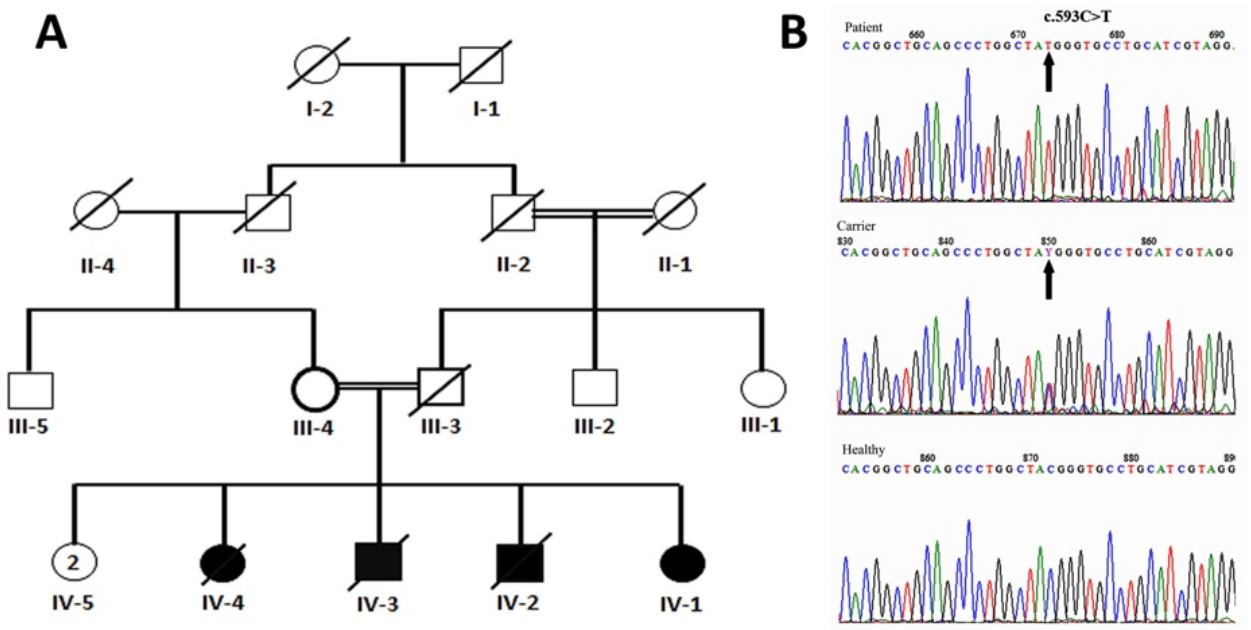

Figure 1: Pedigree and Sanger Sequencing. **(A)**: The pedigree chart spanning four generations. Squares represent males, and circles represent females. Filled circles or squares indicate affected individuals. A double line between parents (III-3 & III-4) signifies a cousin marriage. **(B)**: Sanger sequencing results. The top panel shows the sequencing results of the patient (IV-1), while the middle and lower panels display those of the carrier (III-4) and healthy family member (IV-5). The arrowhead indicates the position of the mutation.

In the current genetic study, the *VSIR* identified homozygous variant may disrupt VISTA's normal function which lead to abnormal T cell activation regulation which contributes to immunodeficiency. In our patient, the clinical history of recurrent infections, high TLC, and hypogammaglobinemia is consistent with a dysfunctional immune system. Although there were no direct functional studies conducted in this case, the patient's phenotype and the variant's predicted deleterious nature suggest a possible link. Interestingly, the patient exhibited marked T-cell lymphocytosis accompanied by hypogammaglobulinemia, which is somewhat inconsistent for classical lymphopenic combined immunodeficiency, where T-cell reduction is typically expected. This phenotype may reflect immune dysregulation rather than a purely lymphopenic CID, similar to observations in other checkpoint-related PIDs such as *CTLA4* and *LRBA* deficiencies, where T-cell expansion occurs alongside impaired B-cell function.[14] Additionally, the observed moderate reduction in NK cells may contribute to immune dysregulation and heightened susceptibility to infections. These findings suggest that *VSIR* deficiency may represent a novel form of immune dysregulation, distinct from classical lymphopenic CID, highlighting the importance of checkpoint molecules in maintaining immune balance.

VISTA has been prominently identified in its role in a range of cancers and checkpoint-related molecules.[15,16] However, there seems to be a lack of correlation with VISTA's role as an immune checkpoint in cancers and PID. Further investigation is warranted to understand the link between *VSIR*. Molecules such as VSIG-3, a ligand of VISTA are shown to inhibit T-cell function, as shown in recent literature. The use-case of said findings is once again involved in VISTA's role in immunotherapeutic strategies. A direct correlation between these studies and VISTA in infection diseases and autoimmune function is still unknown.[17]

In the current study a key limitation is that we couldn't perform functional experiments to confirm the effect of the identified *VSIR* variant. While computer-based predictions and family segregation analysis suggest it may be harmful (deleterious), we do not have direct evidence showing how the *p.Thr198Met* change affects VISTA function or T-cell regulation. Therefore, the link between this variant and the patient's immunodeficiency should be considered suggestive, and further laboratory studies are needed to confirm its role.

CONCLUSION

This family-based genetic investigation identifies a rare homozygous *VSIR* variant as a potential contributor to immune dysregulation consistent with combined immunodeficiency. The integration of clinical, immunophenotypic, and segregation data supports a plausible role of *VSIR* in T-cell regulation and immune homeostasis, particularly in consanguineous populations. These findings expand the spectrum of candidate genes implicated in primary immunodeficiency disorders. However, functional studies are

required to confirm the pathogenicity of the identified variant and to elucidate the mechanisms linking VISTA dysfunction to T-cell abnormalities and increased susceptibility to infections.

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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

MI, LMA & HRR: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

MB, RN & MH: Acquisition, analysis and interpretation of data, critical review, approval of the final version to be published

SIR: Conception and study design, acquisition, analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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