ORIGINAL ARTICLE

NOVEL HETEROZYGOUS PSMB8 MUTATION IN CANDLE SYNDROME: FIRST REPORT OF ASSOCIATED LOW NATURAL KILLER CELL LEVELS

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Background: Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome is a rare auto-inflammatory inherited type of condition classified in the past few years. The clinical manifestations are observed during the first year of life. Inherited mutations in the *PSMB8* gene are reported to cause CANDLE syndrome. **Methods:** Detailed family and clinical histories were obtained. Genomic DNA was extracted from the whole blood. DNA Sanger sequencing was performed to identify disease-causing mutations in the gene *PSMB8* (Proteasome subunit beta type-8). Complete Blood Count (CBC), Liver Function Test (LFT), blood culture, contrast tomography scan (CT) and ultrasound scrotum were performed. An expert dermatologist investigated nodular erythema or erythematous eruptions. **Results:** DNA Sanger sequencing revealed a heterozygous missense mutation in gene *PSMB8* (c.599C>T). The patient's mother was homozygous for the missense mutation. High-grade episodic fever with low-fat levels (possibly lipodystrophy) was observed in body areas. Blood CBC revealed neutrophilia while the CT scan showed post-infective consolidation in the upper lobe of the posterior segment. **Conclusion:** We report a novel heterozygous genetic mutation. Clinical investigations revealed disease features partially fulfilling the criteria. We also report here CANDLE syndrome with low levels of natural killer cells (NK cells).

Keywords: DNA Sanger sequencing, *PSMB8*, Flow cytometery, NK-cells, CANDLE syndrome

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INTRODUCTION

Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature (CANDLE) syndrome is a rare, inherited auto-inflammatory disease caused by loss-of-function mutations in proteasome components, resulting in proteasomal dysfunction. It is now recognized as a subgroup of proteasome-associated auto-inflammatory syndromes (PRAAS).[1,2] Defective protein homeostasis in CANDLE syndrome leads to intracellular stress caused by the accumulation of polyubiquitinated proteins, triggering inflammation. The disease is strongly associated with an exaggerated interferon response (Type I and Type II). Clinical manifestations of CANDLE syndrome can vary significantly among patients.[3] The common features include erythematous rash, episodes of without identifiable infection called 'sterile fevers', lipodystrophy, musculoskeletal involvement, panniculitis, metabolic derangements, vascular disorders such as systemic and pulmonary arterial hypertension, central nervous system (CNS) disorders.[2,3] Studies related to pathogenesis and treatment revealed the key role of proinflammatory cytokines in amplifying abnormal immune responses can be effective targets for treatment.[4]

Next-generation sequencing (NGS) has been instrumental in identifying a variety of Mendelian defects linked to immune dysregulation and auto-inflammatory diseases. Its application has significantly advanced early diagnosis in paediatric patients presenting with systemic sterile inflammation, highlighting its critical role in modern medical genetics and personalized medicine. Gene mutations in proteasome-associated auto-inflammatory syndromes (PRAAS) are complex, with recent findings recognizing the occurrence of digenic mutations contributing to PRAAS phenotypes. Earlier genetic studies on CANDLE syndrome reported homozygous mutations in the *PSMB8* gene, which encodes the B5i subunit responsible for the chymotrypsin-like enzymatic activity of the immunoproteasome.[3,5] Genetic analysis in CANDLE syndrome patients has previously identified a variety of mutations, both homozygous and heterozygous, in multiple genes, including *PSMB8* (OMIM 177046), *PSMB4* (OMIM 602177), *PSMB9* (OMIM 177045), and *PSMA3* (OMIM 176843).[3,6]

The study was conducted to identify genetic variants associated with CANDLE syndrome in a six-year-old male patient through comprehensive genetic and clinical investigations.

METHODOLOGY

The project titled 'inborn rare genetic diseases in Pakistani population' was designed at HBS Medical and

Dental College Islamabad. Approval was obtained from the Institutional Review Board (IRB-HBSM&DC). Written and verbal consent was obtained from all the participants including patient, parents, and healthy siblings.

A six-year-old male patient from a remote village of Punjab was brought to the Dermatology Department of HBS General Hospital, Islamabad. Patient's complaints included skin lesions along with mild fever almost every morning. Detailed clinical and family history was obtained by expert dermatologist and a paediatrician. Laboratory tests including blood complete picture, and lymphocyte subset analysis were done. The mother of the patient informed physicians that one elder sister (age 8 years) and a maternal cousin (age 10 years) is also suffering from a similar type of disease. An expert molecular biologist drew a family chart. Based on family and clinical history, genetic analysis was done.

A whole blood sample (5 mL) was obtained from patient's (P1 and P2) to perform lymphocyte subset analysis, Complete Blood Count (CBC) and C-reactive protein (CRP). After about a week of initial laboratory tests, another 3 mL whole blood samples were collected in EDTA blood tubes (Becton, Dickinson and Company (BD), BD Global Headquarters USA) from patients (P1 and P2), mother (Pedigree number III-3), and healthy sibling (IV-2) to perform genetic analysis. Genetic analysis was not performed in Patient P3 (pedigree number IV-4) as he was not available.

A blood complete picture test was performed on both patients (P1 and P2) using Sysmex XP-100 machine (Sysmex Corporation, Chuo-ku, Kobe 651-0073, Japan) located in the Department of Pathology HBS General Hospital, Islamabad. Using a flow-cytometer (BD Biosciences, San Jose, CA, USA) lymphocyte subset analysis was performed following the protocol mentioned reported earlier.[7]

Genomic DNA was extracted from WBCs using GenEluteTM blood genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA). DNA concentration was measured using a Nanodrop1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA). Based on clinical investigation (mainly lipodystrophy) CANDLE syndrome was suspected. Primers were constructed for disease-causing genes using PRIMER 3 software (http://bioinfo.ut.ee/primer3-0.4.0/). Polymerase Chain Reaction (PCR) was performed in (25 µL volume) following the standard protocol. A commercially available automated PCR kit (Axygen, CA, USA) with ready-made master mix was used. The PCR-amplified products were purified using 70% ethanol. The purified PCR products were subjected to Sanger sequencing using BigDye Terminator V3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA sequencing was conducted by Alpha Genomic, Islamabad, Pakistan.

The data obtained through Sanger sequencing of the *PSMB8* gene from patients and available parents and a healthy control sample was compared with the corresponding healthy DNA sequence obtained from the Ensemble Genome Browser database (http://ensembl.org/index.html). Mutational analysis was conducted using nucleotide sequence variant Bio Edit sequence alignment editor version 6.0.7. Two bioinformatics software Phenotyping V2 (PolyPhen 2) and MutationTaster (http://www.mutationtaster.org/) were used to measure the disease-causing ability of the identified variants.

RESULTS

We enrolled two patients a 6-year-old male child and a female child of 10 years (IV-1 and IV-3) from a highly consanguineous family. The male patient is second in birth after a healthy male child. The male child was brought to the hospital exhibiting lipodystrophy, erythematous body rashes on the neck, legs, and arms, sterile fevers, and panniculitis as shown in figure (Figure-1 C–F). Supportive care along with symptomatic relief was immediately provided to manage severe rashes and panniculitis with cyclosporin, and topical corticosteroids. In patient (P1) ultrasound scrotum revealed Patent Processus Vaginalis (PPV) right with large amount of fluid in the right scrotal sac. These findings are suggestive of Bubonocele (Figure-1 D).

Flow cytometry mediated lymphocyte subset analysis revealed reduced percentage and absolute number of $CD16^{+}/CD56^{+}$ NK cells. Total Leucocyte Count (TLC) were raised while lymphocyte percentage were within in healthy range. While elevated levels of lymphocyte count and low levels of $CD3^{+}$, $CD3^{+}CD4^{+,}$ and $CD3^{+}CD8^{+}$ were observed. $CD16^{+}+56^{+}$ were low while CD4:CD8 was in a healthy range. Detailed values of lymphocyte analysis are mentioned in Table-1. The patient's complete blood count (CBC) was normal, with mild anaemia, haemoglobin (11.2 g/dL and 9.8 g/dL) and, 39% and 41% lymphocytes, 57% and 55% neutrophils, and 4% and 2% monocytes. (Table-1).

According to literature review mutations in four genes that are *PSMB8* (OMIM, 177046), *PSMB4* (OMIM, 602177), *PSMB9* (OMIM, 177045), *PSMA3* (OMIM, 176843) are associated with CNADLE syndrome. Maximum cases of CANDLE syndrome mutations are reported in *PSMB8*. We selected the same gene to sequence at first. We performed DNA Sanger sequencing in one of the patient (IV-1) initially. A novel heterozygous mutation (c.599C>T; Thr200Met) was identified in exon 5 of the gene *PSMB8*. To test the segregation of the identified variant whole family including (III-3, IV-1, IV-2, IV-3) were sequenced (Figure-1A).

Table-1: Patient lymphocyte subset analysis and complete blood count

Peripheral blood lymphocyte subset analysis (P1)			
Parameters		**Results**	**Reference range**
TLC		15,000/µL	6,400–11,000/µL
Lymphocyte Percentage		54%	38–59%
Lymphocyte Count		8,100/µL	2,700–5,400
$CD3^+$ cells		48% (3,888)	58–67% (1,700–3,600)
$CD3^+CD4^+$cells		34% (2,754)	38–50% (1,700–2,800)
$CD3^+CD8^+$cells		13% (1,053)	18–25% (800–1,200)
$CD19^+$ cells		48% (3,888)	19–31% (500–1,500)
$CD19^+CD56^+$cells		**1% (81)**	**8–17%(300–700)**
CD4:CD8		2.6	1.5–2.9
Complete Blood Count (P1, P2)			
Parameters	**P1**	**P2**	**Reference Range**
TLC	6.3×10^9 g/L	6.4×10^9 g/l	4–11 g/L
RBC	4.0×10^{12}/L	3.55×10^{12}/L	4.5–6/L
Haemoglobin	**11.2 g/dL**	**9.8 g/dL**	13–15 g/dL
Platelets	382×10^9 g/L	394×10^9 g/L	150–400
Neutrophils	57%	55%	40–75%
Lymphocyte	39%	41%	20–45%
Monocytes	4%	2%	2–10%
Eosinophils	3%	2%	1–5%

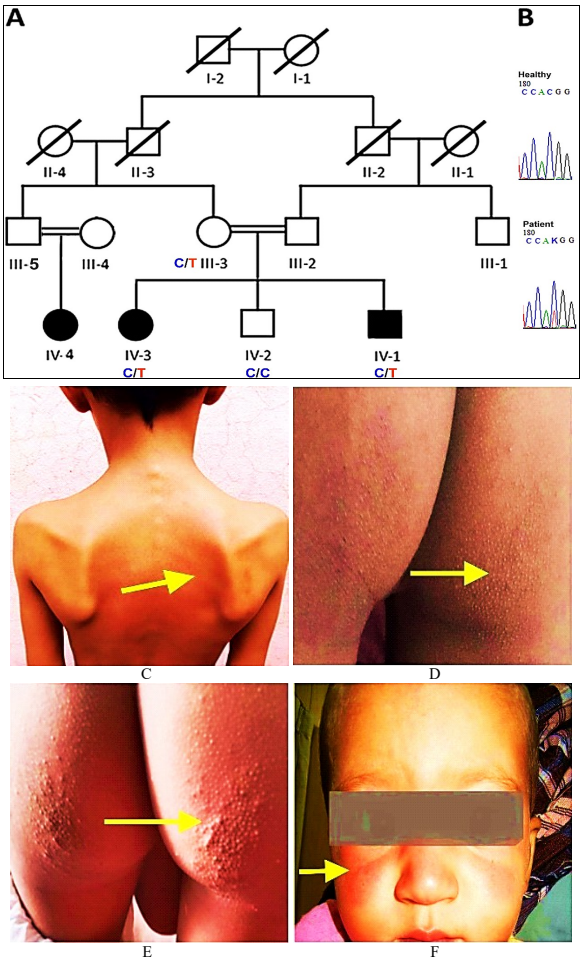

Figure-1 A: Family Pedigree. **Figure-1 B:** DNA sequencing: DNA sequence chromatograms (top panel) represent a healthy sequence while the lower panel shows heterozygous peaks in patients. **Figure-1 C, D & E** represents patient IV-1. Arrow shows lipodystrophy in the back side of the trunk region, while eczema is observed in hip region indicated by arrow head. Patent Processus Vaginalis (PPV) right with large amount of fluid collection in the right scrotal sac in Patient 1. **Figure-1 F** shows red face skin with inflammation in the patient IV-3.

DISCUSSION

Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature (CANDLE) is a rare auto-inflammatory inherited syndrome characterized by epithelial rashes associated with fever and numerous systemic symptoms. Combination of mutations with monogenic as well digenic inheritance in proteasome maturation protein gene *POMP* and proteasome subunit beta type-8 gene *PSMB8* lead to defected proteasome assemble or total deficiency of proteasome and cause CANDLE syndrome.[3,6]

CANDLE syndrome patients exhibit diverse clinical manifestations, including erythematous plaques, purpuric lesions, recurrent inflammation, 'aseptic' meningitis, hepatomegaly, abdominal pain, and skin flare-ups.[2,6,8] Our patients (P1 and P2) reported here presented with sterile fever, lipodystrophy, erythematous body rashes, and panniculitis coherently to those reported previously. Meningitis, hepatomegaly and abdominal pain were not observed in our patients. Laboratory investigations in CANDLE syndrome patients reveal hypochromic anaemia, leukocytosis, increased platelet count, and slightly elevated levels of aspartate aminotransferase.[2,6,8] Laboratory findings in our patients revealed mild iron deficiency anaemia. Reduced levels of NK cells with lymphocytosis was noted in one of our patient (P1). Genetic analysis showed a novel heterozygous mutation (c.599C>T, Thr200Met) in the *PSMB8* gene. The gene *PSMB8* which encodes the B5i subunit containing the chymotrypsin-like enzymatic activity of the immuno-proteasome is located on chromosome 6p21.32.[3,5] The gene encodes for α7 subunit of the proteasome complex. Genetic analysis in patients with CANDLE syndrome reveals homozygous, heterozygous, and heterozygous in combination with digenic mutations such as *PSMA3*/*PSMB8*, *PSMB9*/*PSMB4* or *PSMB8*/*PSMB4*.

MANAGEMENT

Only a limited treatment options are available to manage CANDLE syndrome manifestations. There is no effective and permanent treatment available. Some improvements can be achieved with oral corticosteroids and methotrexate.[8] Dapsone, colchicine, cyclosporine, azathioprine, and etanercept are not helpful. In the current study, NSAIDs were used to manage fever.

CONCLUSION

We identified a rare genetic disorder, CANDLE syndrome, in a family. Functional analysis using flow cytometry revealed a deficiency in natural killer cells, providing insight into the immunological aspect of the disease. A novel heterozygous missense mutation was uncovered, which segregates within the family, contributing to the understanding of the genetic basis of CANDLE syndrome. These findings emphasize the importance of integrating genetic and functional studies to elucidate rare disorders and pave the way for potential diagnostic and therapeutic advancements.

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