

## ORIGINAL ARTICLE

WHOLE EXOME SEQUENCING OF A PAKISTANI FAMILY  
SEGREGATING AUTOSOMAL RECESSIVE GAUCHER DISEASE TYPE I

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**Background:** Gaucher Disease (GD) is a rare inherited lysosomal storage disorder caused by mutations in the *GBA1* gene on chromosome 1q21. This study aimed to perform clinical and genetic evaluation of two patients with clinically suspected Gaucher's disease and to identify the underlying pathogenic *GBA1* variants. **Methods:** Two patients from a consanguineous Pakistani family were admitted with developmental delay and hypotonia. Detailed physical examination was performed. Complete blood count (CBC), Liver Function Tests (LFTs), Inflammatory markers, Erythrocyte Sedimentation Rate (ESR), and radiological procedures including ultrasound and MRI were advised. Later, Whole Exome Sequencing (WES) followed by DNA Sanger sequencing was performed. **Results:** Analysis of complete blood count demonstrated features of microcytic, hypochromic anaemia in the patients. No abnormalities were observed in liver function tests or erythrocyte sedimentation rate. Physical examination revealed hypotonia. Radiological analysis showed hepatosplenomegaly. Genetic analysis identified a homozygous missense mutation, c.1448T>C; p.Leu483Pro, in the *GBA* gene. **Conclusion:** This study confirms Gaucher disease in two Pakistani patients with a homozygous *GBA1* variant and highlights the importance of integrating clinical assessment with genetic testing for accurate diagnosis.

**Keywords:** Bone Marrow, Gaucher Disease, *GBA1* Gene, Genetic Analysis, Haematological Tests

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## INTRODUCTION

Philip Gaucher, a French dermatologist, in 1882, identified an abnormality in a patient with splenomegaly, later named Gaucher cells with condensed chromatin and eccentric nucleus. Later, the disease was termed 'Gaucher Disease'. The Gaucher cells are, in fact, abnormal macrophages, preferential targets of this rare autosomal and recessive genetic disease.<sup>1,2</sup> Gaucher disease (GD) is the most common lysosomal storage disorder worldwide. It is an autosomal recessive condition caused by mutations in the acid  $\beta$ -glucosidase gene (*GBA1*; OMIM 606463) located on chromosome 1q21, resulting in deficiency of the lysosomal enzyme  $\beta$ -glucocerebrosidase.<sup>3,4</sup> This deficiency leads to accumulation of glucocerebroside within lysosomes, particularly in the spleen, liver, lungs, and brain, forming characteristic Gaucher cells.<sup>5–7</sup> GD is classified as a lysosomal sphingolipidosis and is reported as one of the more frequent genetic disorders in certain populations.<sup>5,6</sup>

Based on clinical presentations, severity, and age of onset, GD can be categorized into three subgroups. Type 1 GD with anaemia, chronic and non-neuropathic, and massive hepatosplenomegaly is observed in early adulthood. Type 2 GD is characterized by progressive neurological weakening with hepatosplenomegaly. Type-3 GD is the juvenile form with onset between 2–6 years. The most common presentation of this type is hepatosplenomegaly along

with a moderate level of neurological symptoms. Skeletal deformities are observed with the progression of age.<sup>8</sup>

Gaucher Disease associated with pulmonary involvement is rare but has been reported in limited case series. The literature review revealed only one case of GD with itching.<sup>8,9</sup> In the current study, we report a highly consanguineous Pakistani family segregating a rare type of Gaucher Disease with potential risk for neuronopathic progression. Genetic analysis of the *GBA1* (OMIM, 230800) gene revealed an already known homozygous variant p.Leu483Pro, which is rare in the Pakistani population.

## METHODOLOGY

A 1.5 years old male (P1) and a 3 years old (P2) female patient suffering from GD-type 1 were enrolled in this study. Both patients belonged to consanguineous parents from a remote area of Lahore. The indexed patients were brought to the Department of Paediatrics at the Pakistan Air Force Hospital, Islamabad.

The study was approved by the Ethical Review Committee of HBS Medical College, Islamabad vide No. HBS/IRB/19/25, Fazaia Medical College vide IBD/FMC/1320/01/PHY, M. Islam Medical College vide No. 1/2026/Bio/MIMDC), and Nishtar Medical College Multan vide No. 02/2026/PHY/NMU. Before taking the patients' history, informed written consent was obtained from the father of patients.

The indexed male patient presented yellow spots in the eye and a swollen belly. The mother also complained of body aches and lethargy. During family history, the mother reported that the elder sibling, a 3-year-old female (P2), had similar symptoms. She also shared the laboratory reports previously conducted for P2. The index patient was then evaluated by an expert paediatric team to exclude possible infections, particularly those involving the gastrointestinal tract. A geneticist suspected a case of genetic disease and required all the important information related to family history, patient clinical presentations, and the onset of the disease. A pedigree was generated based on the patients' family history.

The physician initially advised complete blood count (CBC), liver function tests (LFTs), and abdominal ultrasound to evaluate splenomegaly. Based on clinical presentation of hepatosplenomegaly and constitutional symptoms, lymphocyte subset analysis and immunoglobulin levels were included in the diagnostic workup to exclude haematological malignancies and lymphoproliferative disorders. Previous laboratory findings of P2, including CBC and lymphocyte subset analysis, were also reviewed.

For laboratory analysis, 5 mL whole blood samples were collected in EDTA/heparin tubes (Becton, Dickinson and Company, USA) for CBC, flow cytometry, immunophenotyping, and DNA extraction. For LFTs, samples were collected in serum separator (gold-top) tubes. All tests were carried out at Armed Forces Institute of Pathology and Immunology Department, Combined Military Hospital, Rawalpindi. Flowcytometry and liver function tests were performed according to the protocols reported in our earlier publication.<sup>10</sup>

## RESULTS

Lymphocyte subset analysis showed relative lymphocytosis with altered T- and B-cell distribution, while NK cell counts were reduced in both patients (P1 and P2) (Table-1). The complete blood count in the patient (P1) revealed leukopenia, anaemia, and low platelet count in P1 and P2 (Table-2). Moreover, differentials revealed high ESR and reticulocyte count (Table-3). Bone marrow aspirates showed hyperplastic erythropoiesis and depressed myelopoiesis in P1. Megakaryocytes were increased with marked dysplasia in P1 (Table-4). Liver function tests showed mildly elevated ALT and AST, while albumin and bilirubin were within the reference range in patient 1.

Pedigree analysis indicated an autosomal recessive pattern of inheritance (Figure-1A). The affected individuals include two siblings, IV-1 and IV-2. Their parents (III-1 and III-2) are clinically healthy but are likely carriers of the condition. One cousin (IV-3) is unaffected and shows no clinical features of the disease.

Whole-Exome-Sequencing (WES) was performed in the male patient (P1, IV-4). There were many single nucleotide polymorphisms (SNPs), identified in the patients. An interesting pathogenic homozygous missense variant c.1448T>C; p.Leu483Pro was identified in chromosome 1q21 in the gene Glucosylceramidase Beta (*GBA*) (Figure-1B). The segregation of the missense variant was confirmed through DNA Sanger sequencing. Mutations in the *GBA* gene are reported to cause Gaucher's Disease.

**Table-1: Lymphocyte subset analysis [n (%)]**

Test Name	P1	P2	Reference Range
TLC (/ $\mu$ L)	4,500	4,700	4,000–12,000
Lymphocytes (%)	72	70	44–74
CD3+ cells	1,652 (51)	1,554 (48)	2,100–6,200 (53–75)
CD3+ CD4+ cells	875 (27)	939 (29)	1,300–3,400 (32–51)
CD3+ CD8+ cells	518 (16)	485 (15)	620–2,000 (14–30)
CD19+ cells	1,458 (45)	1,198 (37)	720–2,600 (16–35)
CD16+ CD56+ cells	97 (3)	161 (5)	180–920 (3–15)
CD4:CD8	1.6	1.6	1.3–3.0

**Table-2: Blood complete picture**

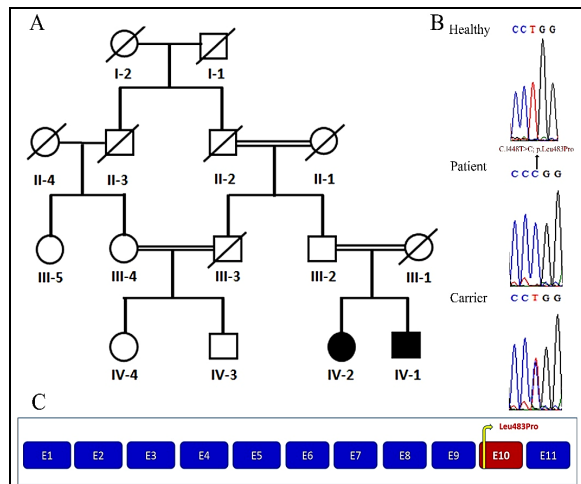
Test	P1	P2	Reference range	
			Male	Female
Total leukocytes ( $10^9/L$ )	4.77	5.51	4.0–10.0	4.0–10.0
RBC count ( $10^{12}/L$ )	3.94	3.18	4.5–6.3	3.8–5.2
Haemoglobin (g/dL)	9.3	8.8	14–18	11.7–15.2
Haematocrit (%)	0.28	0.31	0.39–0.49	0.35–0.48
MCV (fL)	71.8	70.3	77–91	77–91
MCH (pg)	23.5	24.5	26–32	26–32
MCHC (g/dL)	32.7	33.7	32–36	32–36
Platelet counts ( $10^9/L$ )	40	48	150–400	150–400
Absolute Neutrophil count ( $10^9/L$ )	0.95	0.97	1.5–7.7	

**Table-3: Differential analysis of blood**

Test	P1	Reference range
Neutrophils (%)	20	40–75%
Lymphocytes (%)	75	20–45%
Monocyte (%)	4	2–10%
Eosinophils (%)	1	1–6%
ESR (mm/1 <sup>st</sup> Hour)	59	(Male: 0–15, Female: 0–20)
Reticulocytes	4.5	(0.5–2%)
<b>Red Cell Morphology</b>		
Anisocytosis		-
Microcytosis		+
Poikilocytosis		+
Macrocytosis		-
Hypochromia		-
NRBCs		1/100 WBCs

**Table-4: Bone marrow aspiration analysis**

Site (s)	Posterior superior iliac spine
Consistency of bone	Normal
Cellularity	Normal Cellular Marrow
Erythropoiesis	Hyperplastic, Normoblastic
Myelopoiesis	Depressed
M.E Ratio	<1
Blasts	Nil
Megakaryocytes	Increased with marked dysplasia
Lymphocytes	Increased 60%
Plasma cells	Normal
Abnormal cells	A few scattered storage cells with crumpled tissue paper appearance seen



**Figure-1: A: represent pedigree chart. Filled circles and squares represent female and male patients, while a double line indicates cousin marriage. B: represents Sanger sequencing of the target patient. Arrowhead showing site of mutation c.1448T>C; p.Leu483Pro. The mutation is segregating in healthy individuals and carriers. C: *GBA* gene structure showing 11 exons with site of mutation in exon 10**

## DISCUSSION

In humans, inherited Gaucher Disease type I (GD1, OMIM 230800) is a relatively common metabolic disease related to lysosomal storage caused by mutations in the *GBA* gene. *GBA* gene deficiency reveal variety of disease phenotypes which are grouped into type 1 (Non-neuronopathic), type 2 (Acute neuronopathic), and type 3 (Chronic neuronopathic). Data on Gaucher disease prevalence in Pakistan are limited, with higher occurrence observed in consanguineous families. In general population the incidence rate is ~1.5 per 100,000 live births, while specifically in the Pakistani population (Selected population) the frequency of GD is 34.4%.<sup>11,12</sup> The gene *GBA* encodes a 497 amino acid protein, glucocerebrosidase (GCCase), a key enzyme required for the breakdown of glucocerebroside, a cell membrane lipid.<sup>13</sup> To date, more than 300 point mutations, large deletions, and insertions have been reported in the gene *GBA*.<sup>14</sup>

The severity of the disease manifestations depends on the type of mutation. Hence, the genetic makeup and the severity of the disease in GD patients are complex genotype–phenotype correlations. In a few cases, patients with similar mutations may exhibit different symptoms of GD. Genetic, Epigenetic, and environmental factors may influence disease expression.<sup>15,16</sup>

In the current study, we performed genetic analysis in two patients suffering from GD belonging to a highly consanguineous Pakistani family. Whole

Exome Sequencing revealed a missense mutation converting nucleotide Cytosine to Thymine at position 1448, resulting in a change of amino acid Leucine to Proline 483 [c.1448T>C; p.Leu483Pro]. This *GBA* gene mutation in the Iranian population was recently reported by Mozafari *et al*<sup>17</sup> and Youssef *et al*<sup>18</sup>. In earlier reports, *GBA* gene variant p.Leu483Pro was reported in both homozygous as well as compound heterozygous states with type 2 and type 3 GD, respectively.<sup>18</sup> In GD patients, the genotype-phenotype correlation is not absolute. The GD patients with the p.Leu483Pro mutation require close monitoring owing to their subtle course and potential late neurological disease phenotypes. In our patients, we observed hepatosplenomegaly and cytopenia with currently no neurological manifestations. In most cases, the neurological manifestations develop late in life. Long-term follow up reveal the development of supranuclear gaze palsy and cognitive impairment. The late onset of neurological manifestations is also observed in patients with enzyme replacement therapy.<sup>19</sup>

The gene *GBA* has approximately 7.6–8 kb of genomic sequence and comprises 11 exons. The encoded protein GCCase comprises three major domains: domain-I, domain II, and domain III. The mutation identified in the current report is located in exon 10 of the *GBA* gene. This mutation lies in domain II of the protein and is predicted to affect proper protein folding, stability, and protein–protein interaction. Like other *GBA* mutations, p.Leu483Pro is also associated with neurological manifestations linked to GD with increased risk of Parkinson’s disease.<sup>20</sup>

## CONCLUSION

This study reports two consanguineous Pakistani patients with Gaucher disease associated with the p.Leu483Pro mutation, which carries a potential risk for neuronopathic progression. Both patients presented with hepatosplenomegaly and cytopenia. These findings broaden the genetic and clinical spectrum of Gaucher disease in Pakistan and highlight the importance of molecular diagnosis and long-term follow-up to monitor for possible late-onset neurological involvement.

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Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

**MI:** Laboratory data acquisition and analysis (Blood sampling)

**MS:** Manuscript writing, data analysis (Blood sampling)

**NS:** Manuscript preparation, data analysis and Sanger sequencing analysis

**AM:** Laboratory work (Immunological) and Data analysis

**SKA:** Critical review and Clinical evaluation

**SA:** Clinical investigation

**SIR:** Critical review and Final approval for submission

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