ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Clinical Manifestations and Genetic Findings in Three Patients with Chediak-Higashi Syndrome: Highlighting the Splice Site Variants

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Received: 19 August 2025; Received in revised form: 23 September 2025; Accepted: 25 September 2025

ABSTRACT

Chédiak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by severe immunodeficiency, partial albinism, recurrent infections, and progressive neurologic dysfunction. Unless patients undergo successful hematopoietic cell transplantation (HCT), a majority of them die during childhood because of an accelerated phase of immune dysfunction and hemophagocytic lymphohistiocytosis (HLH). Herein, we aim to describe the laboratory diagnosis, clinical manifestations, and genetic findings in three patients with CHS.

Three patients with partial albinism associated with CHS were included in this study. Immunological screening tests were done. Leukocyte granules in peripheral blood smear (PBS) and hair shafts were examined. Subsequently, genetic analyses were performed by Whole Exome Sequencing (WES) followed by Sanger sequencing for all patients and their parents.

Initial immunology screening tests were within normal limits. Light microscopy studies of patients' PBS showed giant granules in the leukocyte cytoplasm. Furthermore, there were evenly distributed melanin granules in the patients' hair shafts. Variant analysis of the *LYST* gene identified three splicing site defects: one known variant, c.7060-1G>A in intron 24, and two novel variants, c.2363+2T>C in intron 5, and c.10702-3A>G in intron 47.

Hair shaft assay and PBS are rapid and suitable tests in early diagnosis and differentiation in CHS patients. WES results revealed 2 novel splice site variants that might contribute to earlier and more accurate diagnosis. This facilitates early enrolment of CHS patients in the HCT protocol-the optimal treatment path-and enables prenatal diagnosis for affected families.

Keywords: Chediak-Higashi syndrome; Inborn errors of immunity; *LYST* gene; Novel variant; Splice Site; Primary immunodeficiency; Whole exome sequencing

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INTRODUCTION

According to the classification of inborn errors of immunity (IEI), collected by the International Union of Immunological Societies (IUIS), Chédiak-Higashi syndrome (CHS) belongs to the diseases of immune dysregulation, the subgroup of familial hemophagocytosis with hypopigmentation.[1-4] CHS is a rare autosomal recessive lysosomal disorder caused by variants in the *LYST* gene. This gene encodes the lysosomal trafficking regulator, a highly conserved 429 kDa cytoplasmic protein, the exact function of which is yet to be elucidated.[4-6] It consists of 53 exons and is responsible for sorting endosomal resident proteins into late endosomes.[4,5,7] Variants in this gene disrupt normal cell functions by causing grossly enlarged and nonfunctional lysosomes, identified as giant granules in granulocyte and monocyte cells.[2,4,5] Patients with CHS often exhibit hypopigmentation of their skin, eyes, and hair, prolonged bleeding times, recurrent infections, abnormal natural killer cell function, and peripheral neuropathy. In about 85% of CHS cases, patients develop a so-called accelerated phase with poor prognosis, which is characterized by high fever, pancytopenia, and hemophagocytic lymphohistiocytosis (HLH).[4,8,9]

Individuals with CHS are susceptible to various bacterial, viral, and fungal infections, especially those affecting the skin and respiratory tract. These infections are often serious or life-threatening and affect CHS patients, leaving them vulnerable to infections throughout their lives, even when they reach adulthood.[4,7,10] Patients surviving childhood are subjected to progressive neurological deterioration such as central and peripheral neuropathies, sensory loss, cerebellar ataxia, and muscle weakness.[2,4,11-13]

Clinical severity can be correlated with molecular genetic findings. In general, null alleles are associated with severe, infantile-onset phenotypes, while missense variants tend to cause a later onset and attenuated form of the disorder.[5,6] Herein, we aim to describe the clinical presentation and molecular analysis of three Iranian patients with CHS.

MATERIALS AND METHODS

Three patients from three unrelated families with oculocutaneous hypopigmentation, recurrent fever and infections, pancytopenia, and hepatosplenomegaly were referred to the Immunology, Asthma and Allergy Research Institute. After clinical evaluation and laboratory investigation, including peripheral blood smear (PBS) and hair shaft study, they were identified as CHS patients.

Peripheral blood samples (1 mL) were collected in EDTA tubes, and hair shaft samples were obtained from the patients' scalps. The giant granules of leukocyte cells in peripheral blood smears and the features of variation in melanin of the hair shaft were determined by light microscopy. Initial immunology screen tests (immunoglobulin analysis, NBT and DHR assay, and complement evaluation) were done for all patients.

Informed consent was obtained from the patient and/or their parents before entering the study.

Molecular Analysis (Whole Exome Sequencing)

Whole Exome Sequencing (WES) libraries were prepared using TruSeq Exome Kit (Illumina, San Diego, CA, USA) targeting all 53 exons of the *LYST* gene. Sequencing was performed on an Illumina NextSeq500 (San Diego, CA). All candidate variants were validated in the patients and their parents by Sanger sequencing.

Different prediction tools were used to assess these novel variants, such as ClinVar. Besides, the Varsome tool was used for the interpretation of the results, and finally, the American College of Medical Genetics (ACMG) guideline was considered for the interpretation of sequence variants.[14]

RESULTS

Clinical Presentation

Three male patients with CHS, aged 4, 7, and 26 years old, were included in the study. The patients' hair and eyebrow color were from gray to light brown. The result of the light microscopy of the scalp hair shaft showed evenly distributed melanin granules and the presence of giant granules in the cytoplasm of leukocytes (Figure 1). Consanguinity is depicted in the pedigrees of the three families (Figure 2).

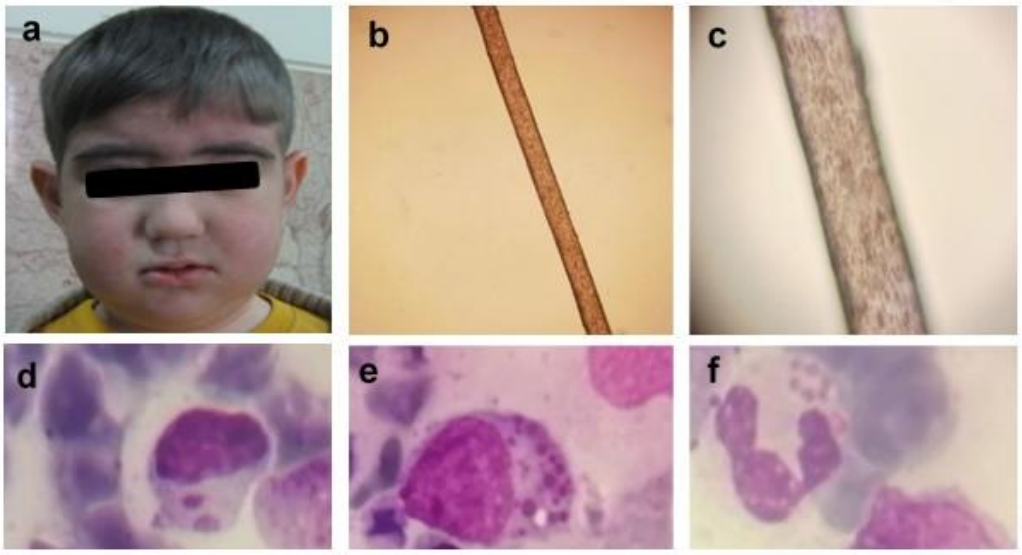

Figure 1. Appearance and microscopic hair study and peripheral blood smear assay in CHS patient (P2). Patient has characteristic gray hair color (a) with evenly distributed pigmentation in hair shaft shown by microscopic examination 10x (b) and 40x magnification (c). Peripheral blood smears (Giemsa stain, 100x) show the presence of giant granules in the cytoplasm of lymphocyte (d), monocyte (e), and neutrophil cells (f).

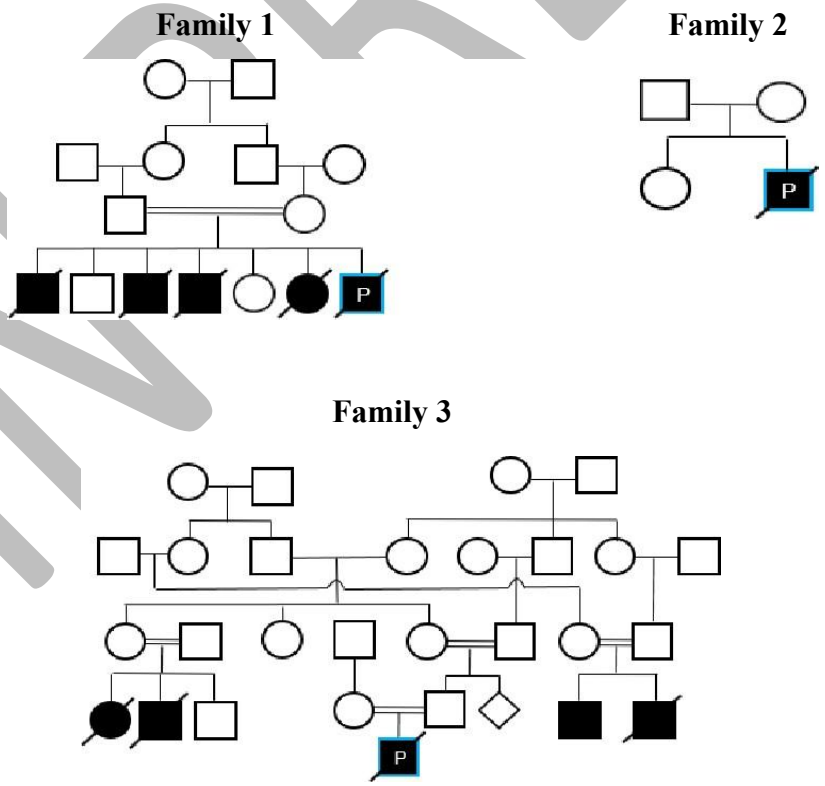

Figure 2. Pedigrees of the three families with CHS. The patterns of inheritance are autosomal recessive in the 3 families. Index cases of the present study are highlighted in blue. There is consanguineous marriage in 2 patients' parents (families 1 and 3) and no consanguinity in family 2. There were some deaths in families 1 and 3 with no definite diagnosis.

A history of recurrent fever and infection with frequent hospitalizations, pancytopenia, and organomegaly was observed in all patients. Patient 1 (P1) was a 26-year-old male with consanguineous parents and a family history of four deceased siblings with HLH (all were above 10 years). He developed recurrent fever, motor impairment, pancytopenia, hepatomegaly, splenomegaly, cytopenia, severe gingivitis, and neutropenia at 24 years of age. Patient 2 (P2) was a 4-year-old boy from non-consanguineous parents. He developed BCG adenitis, recurrent fever, pancytopenia, and HLH from 36 months of age. Patient 3 (P3) was a 7-year-old boy from consanguineous parents. He was hospitalized at the age of 6.5 years due to pneumonia, hepatosplenomegaly, and HLH. Three affected family members died due to similar clinical manifestations; however, they had no definite diagnosis (Table 1).

Unfortunately, all patients in this study died due to the development of HLH and a lack of a suitable donor for HCT (Table 1).

Immunological Laboratory Findings

Immunological screening tests, including serum immunoglobulin levels (IgM, IgG, IgA, and IgE), complement assay (CH50, C3, and C4), DHR assay, and NBT slide test, were in the normal range.

Genetic Results

Whole exome sequencing results revealed three splicing site defects in the *LYST* gene of these patients, including one known variant c.7060-1G>A in intron 24 and two novel variants c.2363+2T>C in intron 5 and c.10702-3A>G in intron 47 (Table 1).

The variant c.2363+2T>C in P1 is located at the second position of the donor splice site at the 5' end of intron 5. Variant c.7060-1G>A in P2 inserts the whole sequence of intron 24 into the mRNA, resulting in a larger mRNA. This can produce a truncated and unstable protein. In P3, we uncovered the variant c.10702-3A>G in intron 47. These variants were confirmed in 3 patients and their parents by Sanger sequencing (Figure 3).

Table 1. Clinical and molecular findings in 3 patients with Chediak-Higashi syndrome

Patients	Sex	Age, y	Age at onset, y	Age at diagnosis, y	Age at HLH onset, y	Clinical manifestations	Nucleotide changes in *LYST* gene	ACMG criteria	ACMG classification
P1	M	26	24	25.5	24	Partial albinism, recurrent fever, gingivitis, developmental delay, HLH	c.2363+2T>C	PVS1, PM2	Likely pathogenic[a]
P2	M	4	3	4	3	Partial albinism, recurrent fever, HLH	c.7060-1G>A	PVS1, PM2	Likely pathogenic
P3	M	7	6.5	7	6.5	Partial albinism, recurrent fever, respiratory infection, HLH	c.10702-3A>G	PM2, PP3	VUS[a]

[a]Novel variant. [b]ACMG: American College of Medical Genetics; CHS: Chediak-Higashi syndrome; HLH: hemophagocytic lymphohistiocytosis; M: male; P1: patient 1; P2: patient 2; P3: patient 3; VUS: variant of uncertain significance; y: year.

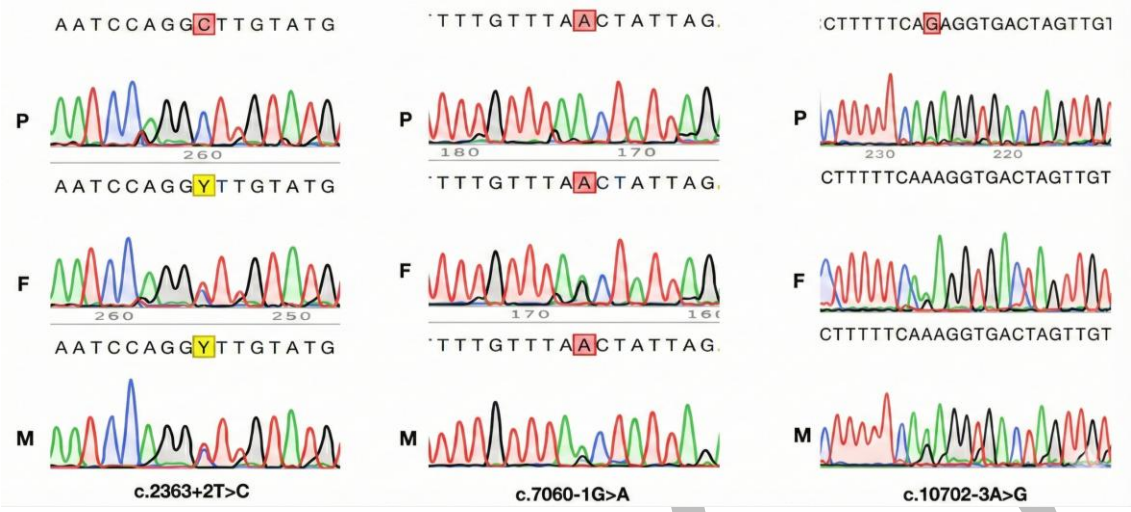

Figure 3. Sanger sequencing of the *LYST* gene in 3 CHS patients and their parents' samples. Sanger validation and segregation analyses variants include one reported variant c.7060-1G>A in intron 24 (family 2) and two novel splicing site defects: c.2363+2T>C in intron 5 (family 1) and c.10702-3A>G in intron 47 (family 3). P: Patient, F: Father, M: Mother

These variants were evaluated by bioinformatics tools such as Varsome, Clinvar, and Franklin.[15] According to these tools, they are likely disease-causing and affect protein function. Finally, the ACMG guidelines were considered for the interpretation of the variants (Table 1).

DISCUSSION

Chediak-Higashi syndrome (CHS) belongs to a large group of inborn errors of immunity (IEI) with partial albinism, the most important of which include Griscelli syndrome type 2 (GS2), Hermansky-Pudlak syndrome type 2 (HPS2), and HPS type 10 (HPS10).[2,4,16-19] CHS is a rare autosomal recessive disorder with an unknown prevalence. The rarity of the condition makes it challenging to establish an accurate prevalence, and many patients likely remain undiagnosed due to the variability in clinical presentation. The syndrome is clinically diagnosed by recognizing giant granules in neutrophils, eosinophils, and granulocytes through light microscopy of a blood smear.[4,16,20]

Microscopic studies for all patients revealed giant granules in neutrophils and lymphocytes or monocytes in PBS and evenly distributed pigmentation in the hair shaft. In the present study, one patient (P1) died with severe symptoms of infection and hepatosplenomegaly and HLH at the age of 26 years, whereas he did not express any serious symptoms of the CHS until the age of 24 years. The age range for individuals with CHS can therefore vary, but the majority of cases present in early childhood, and the condition is generally fatal at a young age.[2] However, some patients with atypical CHS can survive more than 20 years.[2,4,21,22] The life expectancy for CHS patients varies depending on the severity of the condition and the presence of complications. The most common causes of death for individuals with CHS are recurrent infections and the development of an accelerated phase (HLH), which results in a lymphoma-like illness.[2,4,19,22,23] The accelerated phase is characterized by fever, hepatosplenomegaly, pancytopenia, and hemophagocytosis.[4,8,19] All patients suffered from HLH in this study.

Developmental delay is a recognized feature of CHS, with patients exhibiting progressive neurologic abnormalities that can include peripheral neuropathy, cerebellar ataxia, seizures, cranial nerve palsies, and developmental delay.[4,6] However, only P1 presented developmental delay in our study. His older age compared to the other patients is consistent with previous studies that defined neurological defects in CHS patients mostly in adulthood.[2,4,6]

Finally, genetic testing of the *LYST* gene confirms the diagnosis. When multiple candidate genes must be evaluated, WES is the most efficient strategy. CHS results from biallelic *LYST* variants, either homozygous or compound heterozygous pathogenic mutations.[24]

In this study, variant analysis of the *LYST* gene identified three splicing site defects, including one known variant c.7060-1G>A and two novel variants

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c.2363+2T>C and c.10702-3A>G, causing severe and adolescent forms of CHS, expanding the genetic spectrum, and contributing to the genotype-phenotype correlation. These splicing variants in the Iranian population were not reported on the Iranome database. However, in a comprehensive review on the *LYST* gene, 12 splicing site defects were reported, including 2 variants of uncertain significance (VUS), 5 likely pathogenic, and 5 pathogenic.[5] In the present study, 1 VUS and 2 likely pathogenic variants were found in 3 splice site variants.

In conclusion, a light microscopic examination with giant granules in PBS and abnormal pigmentations in the hair shaft of IEI patients with partial albinism can be suspected as CHS. However, while the PBS and hair study are very helpful methods, there is a possibility of technical diagnostic error; final confirmation of any disorder, including CHS, should be done by molecular analysis. The two novel splice site variants of the *LYST* gene reported in this study can complete genetic databases such as HGMD or OMIM. The findings of this study can benefit the early and timely diagnosis of CHS and might help with prenatal diagnosis in the affected families, and raise awareness. Genetic counseling may be beneficial for CHS patients and their families to understand the genetic implications of the condition. HCT is considered the most successful treatment for CHS, particularly if performed before the accelerated phase in the early-onset form of the syndrome.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of Immunology, Asthma and Allergy Research Institute (IR.TUMS.IAARI.REC.1399.022).

FUNDING

The financial support for this study was provided by Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences (No. 95-3-95-51444) and France's National Research Agency (Agence Nationale de Recherche; ANR), the Investment for the Future Program (Programme des Investissements d'Avenir; PIA) through Strasbourg's Interdisciplinary Thematic Institute (ITI) for Precision Medicine, TRANSPLANTEX NG, as part of the ITI 2021-2028 Program of the University of Strasbourg, CNRS and INSERM, funded by IdEx Unistra [ANR10-IDEX-0002] and SFRI-STRAT'US[ANR-20-SFRI-0012].

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

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

AI ASSISTANCE DISCLOSURE

No artificial intelligence tools were used in the preparation of this article.

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