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ANTICIPATORY DETECTION OF LIG4 SYNDROME: CONTRIBUTION OF GENETIC DIAGNOSIS IN A HIGH-RISK SIBLING GROUP

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ABSTRACT
LIG4 syndrome is a rare autosomal recessive DNA repair disorder characterized by microcephaly, facial dysmorphism, combined immunodeficiency, and bone marrow failure. Early diagnosis is essential for timely therapeutic intervention. We report two Moroccan siblings with LIG4 syndrome in a retrospective descriptive study. The older brother, confirmed by whole-exome sequencing, presented with developmental delay, dysmorphic features, recurrent infections, pancytopenia, and died at 14 years. Genetic analysis revealed compound heterozygous variants in the LIG4 gene (c.1271_1275del and c.847A>G). The younger brother, evaluated phenotypically at 7 months, showed growth retardation, microcephaly, dysmorphic face, combined immunodeficiency, and pancytopenia. He is currently awaiting hematopoietic stem cell transplantation from a compatible sibling donor. These cases illustrate the clinical variability of LIG4 syndrome and underscore the importance of anticipatory genetic diagnosis in affected families to guide early management and genetic counseling.

KEYWORDS: LIG4 syndrome; DNA repair disorder; combined immunodeficiency; microcephaly; pancytopenia; genetic diagnosis.

INTRODUCTION
LIG4 syndrome is a rare genetic disorder caused by mutations in the *LIG4* gene, which encodes DNA ligase IV, a key enzyme in the repair of DNA double-strand breaks through the non-homologous end joining (NHEJ) pathway.[1,2] This defect leads to genomic instability responsible for a multisystemic phenotype characterized by microcephaly, neurodevelopmental delay, typical facial dysmorphism, combined immunodeficiency, and progressive bone marrow failure.[3,4] Due to the severity and multisystemic nature of the syndrome, early recognition is essential to allow curative treatment through hematopoietic stem cell transplantation (HSCT) before the onset of severe complications.[4,5] The recent development of whole-exome sequencing has facilitated rapid molecular diagnosis, enabling early detection of affected patients and at-risk relatives.[7] Herein, we report two familial Moroccan cases of LIG4 syndrome, with exome sequencing confirming the diagnosis in the older brother, to illustrate the importance of early detection in high-risk sibling groups.

METHODS
We conducted a retrospective descriptive study in the pediatric department of the Mohammed V Military Instruction Hospital (HMIMV) in Rabat, with parental consent and within the framework of a molecular genetics research protocol. **Case 1** Whole-exome trio sequencing (patient and both parents) was performed at IntegraGen Genomics, using the Agilent SureSelect All Exon V5 capture kit and Illumina HiSeq 2000 sequencing platform (average coverage depth: 75X). Variants were filtered based on allele frequency (variants with frequency >0.5% excluded), genomic location (exonic and splice-site regions), and predicted pathogenicity (using PolyPhen-2 and MutationTaster). Variant confirmation and familial segregation analysis were carried out by the Department of Genetics, Robert Debré Hospital, Paris, France, using Sanger sequencing.. **Case 2**, the younger sibling, was clinically and biologically followed starting at 7 months of age. Complementary investigations included a complete hematological workup, lymphocyte immunophenotyping

(CD3, CD4, CD19), immunoglobulin dosage, bone marrow biopsies, and a standard karyotype. Brain and abdominal imaging were performed to screen for associated anomalies.

RESULTS

Clinical Presentation

Case 1 – Older Brother

The patient, a male child, was born at term in Morocco to non-consanguineous parents, with a birth weight of 1.5 kg. Family history revealed the death of a sister at 18 months in a context of pancytopenia. Gross motor development was partially preserved: standing at 6 months and independent walking by 12 months. However, he exhibited severe language delay, with first words appearing around age 6. He had moderate intellectual disability and was able to perform basic tasks such as eating and dressing.

The clinical picture included chronic diarrhea, recurrent respiratory infections, and early-onset hemorrhagic syndrome. At age 9 years, he was hospitalized, and severe pancytopenia was diagnosed, requiring repeated blood transfusions.

At 10 years and 2 months, physical examination revealed marked growth retardation (height: 102 cm, −5.7 SD), severe microcephaly (head circumference: 44 cm, −6.4 SD), and characteristic facial dysmorphism: triangular face, large eyes with slightly down-slanting palpebral fissures, prominent nose with low-set columella, hypoplastic nasal wings, short philtrum, and moderate retrognathia. Multiple warts were observed on the limbs. Neurological examination was normal. The patient subsequently died from progressive bone marrow failure at 14 years.

Case 2 – Younger Brother

Imad was born at term with intrauterine growth restriction (IUGR) and a birth weight of 2 kg. Growth retardation and microcephaly were noted by 7 months of age. Facial dysmorphism included a bird-like face, prominent forehead, and large eyes. At age 2, he developed a severe hemorrhagic syndrome (epistaxis, melena) associated with pancytopenia, in the absence of infections or tumor infiltration. Bone marrow biopsy was normal.

Hematological assessment revealed severe anemia (Hb 7.5 g/dL), profound thrombocytopenia (platelets 8,000/mm³), and neutropenia. Immunophenotyping showed immunodeficiency combined with T-cell (CD3+, CD4+) and B-cell (CD19+) lymphopenia, and IgG4 subclass collapsed while the total IgG4 remains normal. Abdominal and brain imaging were normal, and the standard karyotype showed no chromosomal instability.

Tableau I: Evolution of the lymphocyte phenotype in the younger brother (absolute counts,percentage, and refrerence ranges).

Parameter	Reference Range (absolute counts)	28/02/2023	27/12/2023
Total lymphocytes		2830	1050
CD3+ (total T cells)	1600–6700	2151 (76%)	788 (76%)
CD3+CD4+ (T helper cells)	1000–4600	821 (29%)	263 (25%)
CD3+CD8+ (cytotoxic T cells)	400–2100	736 (26%)	452 (43%)
CD3+CD5+		58%	67%
CD3+CD4+CD8+		3%	2%
CD3+CD4−CD8−		18%	6%
CD3+CD4+CD7−		15%	8%
CD3+CD4−CD7−		22%	35%
CD3+CD4+CD56+		18%	1%
CD3+CD8+CD56+		17%	1%
CD4/CD8 ratio		1.12	0.58
Natural Killer cells (CD3−CD56+)	200–1200	113 (4%)	74 (7%)
B lymphocytes (CD19+)		105 (20%)	105 (10%)
CD19+CD20+		105 (19%)	105 (10%)

Tableau II: HLA typing of the recipient (Imad) and the familial donor (Khadija, sister born 01/01/2014).

Locus	Recipient (Imad)	Donor (Khadija)
HLA-A	A*01*, *A*24	A*01*, *A*24
HLA-B	B*39*, *B*50	B*39*, *B*50
HLA-C	*C06*, *C*07	*C06*, *C*07
HLA-DRB1	DRB1*03*, *DRB1*08	DRB1*03*, *DRB1*08
HLA-DQB1	DQB1*02*, *DQB1*04	DQB1*02*, *DQB1*04

A clinical diagnosis of LIG4 syndrome was made. Hematopoietic stem cell transplantation is being considered, and an HLA-compatible sibling donor has been identified.

Tableau III: Clinical comparison of two brothers exhibiting clinical similarity in LIG4 syndrome.

Parameter	Case 1 (Older brother, genetically confirmed, deceased at 14 years)	Case 2 (Younger brother, clinically suspected, alive at 25.5 months)
Family relationship	Older brother from a non-consanguineous union	Younger brother from the same non-consanguineous union
Birth weight	1.5 kg (< −3 SD)	2.0 kg (−2.5 SD)
Growth retardation	Present	Present
Infections	Recurrent respiratory infections, chronic diarrhea	Recurrent otitis media, ENT infections
Hematological involvement	Bone marrow aplasia requiring regular transfusions	Moderate to severe pancytopenia
Immunodeficiency	Data not available	Combined immunodeficiency, severe IgG4 subclass deficiency
Facial dysmorphism	Triangular face, hypertelorism, narrow nose	Triangular face, hypertelorism, narrow nose
Skin signs	Warts, telangiectasias, petechial purpura	Pallor, no specific skin lesions
Organomegaly	Data not available	Mild hepatosplenomegaly
Neurological status	Moderate intellectual disability	Moderate intellectual disability
Karyotype / Genetics	Genetically confirmed: c.1271_1275del + c.847A>G	Suggestive family history + identical clinical phenotype
HLA typing for transplantation	Not performed	HLA-compatible sibling donor (Khadija, born 01/01/2014)
Recent clinical course	Deceased at 14 years	Recurrent hospitalizations for epistaxis, pancytopenia, transfusions

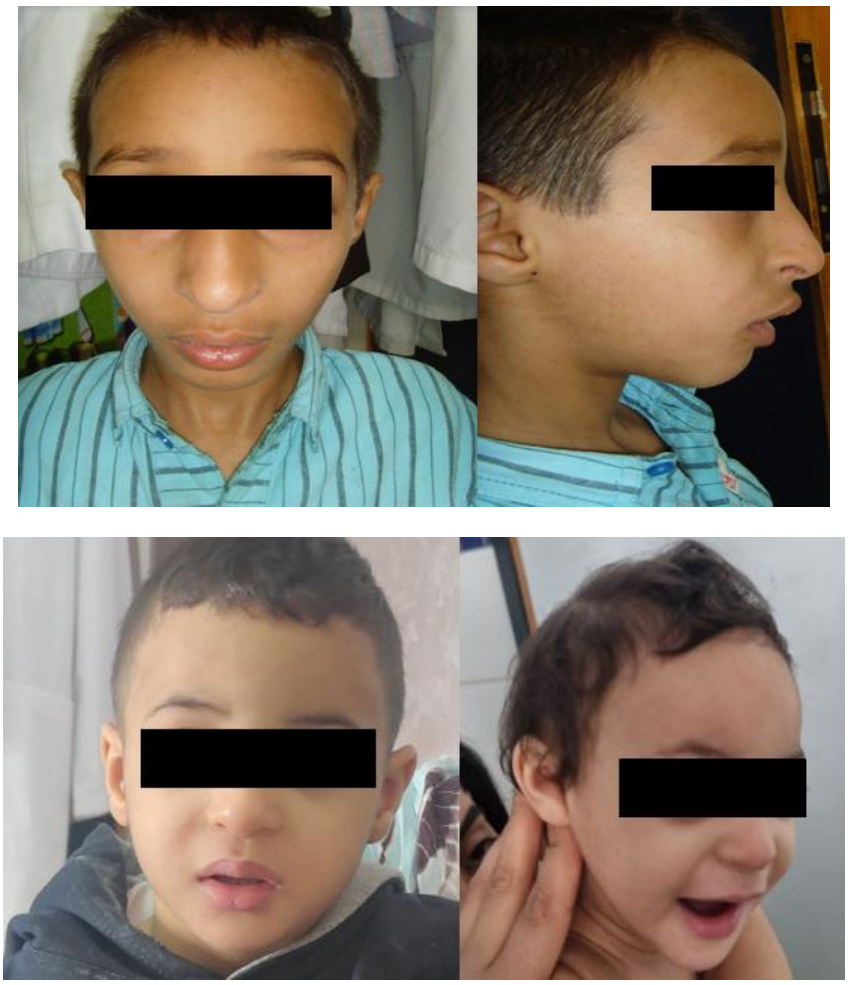

Figure 1: Characteristic dysmorphic features in tow siblings with LIG4 Syndrome.

Molecular Data

Whole-exome sequencing performed in the older brother revealed a compound heterozygous state for pathogenic variants in the *LIG4* gene (NM_206937.1): a 5-base-pair deletion in exon 4 (c.1271_1275del, p.Lys424Argfs*20), previously reported in the literature, and a missense mutation (c.847A>G, p.Lys283Glu), predicted to be deleterious by PolyPhen-2 and Mutation Taster. Familial segregation analysis confirmed parental heterozygosity, establishing the genetic diagnosis.

In the younger brother, the diagnosis is based on clinical, immunohematological, and familial criteria, pending molecular confirmation.

DISCUSSION

LIG4 syndrome is a rare autosomal recessive genetic disorder caused by defective repair of DNA double-strand breaks through the non-homologous end joining (NHEJ) pathway.[1,2] This defect results in genomic instability, giving rise to a multisystemic phenotype including microcephaly, neurodevelopmental delay, characteristic facial dysmorphism, combined immunodeficiency, and progressive bone marrow failure.[3,4]

Phenotypic Variability and Clinical Diagnosis

Our two cases illustrate the broad clinical spectrum of LIG4 syndrome, even within the same family, a variability also reported in the literature.[5,6] The older brother had severe language delay, moderate intellectual disability, and classic facial features (triangular face, large eyes, prominent nose, short philtrum), along with severe pancytopenia and recurrent infections. These manifestations are consistent with the findings of Riballo et al. (1999) and Boone et al. (2014), who describe a clinical spectrum that includes growth retardation, cognitive impairment, and infection susceptibility due to immunodeficiency.[3,7]

The younger brother presented a similar profile, with bird-like facial dysmorphism, combined immunodeficiency, and evolving pancytopenia. Differences in age of onset and severity reflect the phenotypic heterogeneity previously described by Li et al.[8], which complicates early clinical diagnosis and highlights the value of molecular approaches.

Genetic Mutations in LIG4 Syndrome

LIG4 syndrome results from a range of mutations affecting the LIG4 gene, encoding DNA ligase IV, a key enzyme in the NHEJ DNA repair pathway.[1,2] Multiple mutation types have been described, including frameshift deletions, nonsense mutations, and missense variants, each variably impacting enzymatic function and clinical severity.[3–5] The c.1271_1275del (p.Lys424Argfs*20) frameshift deletion identified in our index patient is a well-characterized mutation commonly associated with severe disease phenotypes.[6] Other mutations reported in the literature, such as c.833G>T (p.Arg278Leu) and c.1138A>G (p.Lys380Glu), show variable phenotypic expressivity ranging from mild immunodeficiency to early fatality.[7,8] Functional studies and genotype-phenotype correlations remain challenging due to clinical heterogeneity and variable effects on ligase IV stability and activity.[9] This underscores the critical role of precise molecular diagnosis to guide management and genetic counseling.[10]

Contribution of Genetic Diagnosis

Molecular diagnosis in the older brother revealed compound heterozygous pathogenic variants (c.1271_1275del and c.847A>G) in the LIG4 gene, thereby confirming the clinical diagnosis. Molecular confirmation is crucial, as it guides therapeutic management and enables accurate genetic counseling.[9] Multiple studies emphasize that performing trio whole-exome sequencing in an index case allows rapid identification of causative mutations in rare syndromes such as LIG4, facilitating early screening of at-risk relatives.[10,11] Although genetic testing was only performed in the older brother in our case, it helped direct the monitoring and clinical management of the younger brother, whose diagnosis is based on clinical and immunohematological criteria.

Prognostic Value of Molecular Findings

The combination of the two mutations found in Case 1 is associated with a severe phenotype.[6,20] The missense variant p.Lys283Glu lies within a key functional domain of DNA ligase IV, suggesting significant impairment of enzymatic function. The c.1271_1275del deletion results in a clear loss of function. Although genetic confirmation is lacking for Case 2, the phenotypic resemblance and familial context strongly suggest involvement of the same gene.

Therapeutic Management

Given the progressive bone marrow failure and severe immunodeficiency associated with LIG4 syndrome, hematopoietic stem cell transplantation (HSCT) is considered the curative treatment of choice.[12] Several studies report better transplant outcomes when performed before the onset of severe infections or advanced marrow failure.[13,14] In our case, identifying an HLA-compatible sibling donor allowed curative transplantation to be proposed for the younger brother, highlighting the importance of early familial detection. Additionally, immune management, including close monitoring of opportunistic infections and appropriate prophylactic treatment, remains a key aspect of care.[15] The observed combined immunodeficiency specifically, IgG4 collapse and T- and B-cell lymphopenia supports this need.[16]

Genetic Implications and Family Counseling

The parents in this family are non-consanguineous, showing that LIG4 syndrome can occur in non-related populations. This underlines the need to consider the diagnosis even in the absence of a known family

history.[17] Genetic counseling relies on molecular confirmation to inform parents of recurrence risk and offer prenatal or preimplantation genetic diagnosis for future pregnancies.[18] Monitoring of asymptomatic or heterozygous relatives may also help prevent or detect early clinical manifestations.

Limitations and Perspectives

This study is limited by the absence of genetic confirmation in the younger sibling, which should be addressed in the future to refine diagnosis and management. Moreover, the rarity of reported cases in Morocco and North Africa highlights the need for increased awareness and broader access to molecular diagnostics.[6] The development of national and international cohorts could improve the clinical and genetic understanding of LIG4 syndrome and support the design of targeted and personalized therapeutic strategies.[19]

CONCLUSION

Genetic diagnosis performed in one affected sibling is essential for the anticipatory detection of LIG4 syndrome within a high-risk sibling group. It enables early therapeutic intervention most notably hematopoietic stem cell transplantation and provides a basis for appropriate family genetic counseling.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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