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Genetic Study In a Pakistani Family Reveals Autosomal Recessive Type of Artemis Deficiency

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Abstract

Objective: To perform clinical and genetic investigations in two patients suffering from Artemis deficiency with total deficiency of T^-B^- lymphocytes.

Methods: We enrolled a Pakistani family with one male patient suffering from Severe Combined Immune Deficiency (SCID). Immunological investigations including lymphocyte subset analysis, and serum immunoglobulin levels were performed. Genetic analysis was performed on patients and available parents. Polymerase Chain Reaction (PCR) based amplification and electrophoresis in patients, parents and control was performed.

Results: A detailed clinical investigation revealed mild fever, lower respiratory tract infections, watery diarrhoea, and oral moniliasis from the age of one month. Lymphocyte subset analysis showed T^-/B^- and NK^+ type SCID. Genetic analysis revealed two exon deletions in the Artemis encoding gene (*DCLRE1C,* OMIM 602450).

Conclusion: This study reveals a novel mega deletion mutation in the gene *DCLRE1C,* (two exon deletions) confirmed through PCR-based electrophoresis. The mutation leads to Artemis deficiency $T^-/B^-/NK^+$ type of SCID.

Keywords: CNV repeats, SCID, Artemis deficiency, Lymphocyte subset analysis, Immunoglobulins.

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1. Introduction

Severe Combined Immune Deficiency (SCID, OMIM 601457) is a subcategory of immune deficiencies termed Primary Immune Deficiencies (PIDs). Worldwide, the approximate occurrence is 1 in 50,000 to 100,000 live births. Primarily SCID is caused by genetic mutations in genes that disrupt the standard development of T-lymphocyte (T-cell), B-lymphocyte (B-cell), and Natural Killer cells (NK-cell) [1,2]

These genetic mutations causing SCID lead to increased vulnerability to infections during the infant stage of life usually with failure to thrive which generally results in early mortality in severe cases. Artemis an endonuclease is a 685 amino acid (77.6 kDa.) protein encoded by (DCLRE1C, OMIM 602450) gene that is involved in the healthy development of B- and T-lymphocytes through V(D)J recombination and is a key factor in double standard (dsDNA) break repair by non-homologous end-joining (NHEJ). The gene DNA Cross-Link Repair 1C (DCLRE1C, OMIM 602450) is localized on chromosome 10p13.[3-5]

Mutations in the DCLRE1C gene lead to the autosomal recessive type of SCID characterized by ($T^-B^-NK^+$) immunophenotype. Patients suffering from Artemis deficiency show a broad spectrum of phenotypes extending from SCID to serum antibody deficiencies due to impaired V(D)J recombination. Also, Artemis deficiency patients suffer from exposure to ionizing radiation and alkylating agents.[6,7,8]

Artemis deficiency is the most common type of SCID with radiosensitivity. The majority of Artemis-deficient (lethal form) patients suffering from SCID show radio sensitivity at the cellular level. DCLRE1C genetic mutations that don't fully abrogate the Artemis function (hypomorphic variants) lead to milder phenotypes.[5] According to a recent Iranian report by Abolhassani, (2018), 0.5% of the registered PID cases by the Iranian National Registry of Primary Immunodeficiencies (IPIDR) show DCLRE1C deficiency.[9] Along with other forms of SCID, the mortality rate reaches (57.2%) in Iran [9]. Recently, Fayyaz et al (2024), reported the first two cases of Artemis deficiency in Pakistan.[10]

In the current study, we enrolled a one-month-old boy (age at admission) from Islamabad suffering from recurrent skin infections. This report discusses the detailed clinical as well as laboratory findings and management of the patient. Genetic analysis in the patient revealed a DCLRE1C deletion.

2. Materials & Methods

Ethical statement: Ethical approval for this study was obtained from the research study was obtained from the Institutional Review Board (IRB) of HBS Medical College, Islamabad, Pakistan. Before, acquiring detailed clinical and family history informed written consent was obtained from the parents. Laboratory investigations in patients (III-2 and III-3) were performed. Informed consent was also obtained to report clinical and genetic findings.

Statistical analysis: The current research study is a "family-based genetic association study ". To find the inheritance pattern of the disease considering the shared genetic background among family members, the DNA sequencing data was analyzed.

Patient & Laboratory Investigations

A male patient aged 1 month from a consanguine marriage from Islamabad, Pakistan was admitted to Combined Military Hospital (CMH), Rawalpindi, Pakistan. Detailed clinical, pathological, and immunological investigations were performed by an expert team of physicians. Detailed family history and pedigree (family chart) were constructed by an expert molecular biologist. Based on the patient's clinical history, the physician ordered immunological tests including lymphocyte subset analysis, and serum immunoglobulin levels (IgGs).

Lymphocyte Subset Analysis:

To investigate the immunological status of the patient, lymphocyte subset analysis and serum Immunoglobulin levels were measured. A whole blood sample (5CC) was acquired from the patient using a butterfly in sodium ethylene diamine tetra acetate (Na-EDTA) tubes (BD Vacutainer® EDTA Tubes, Becton Dickinson, UK). Lymphocyte subset analysis was performed through flow cytometry using anti-antibodies (CD19, CD3, CD4, CD8, and CD16/56) from (BD Biosciences, San Jose, CA, USA) following a standard protocol as discussed in our recently published study [11]. The test was performed on a BD FACSCanto™ II Clinical Flow Cytometry System (Becton Dickinson, San Jose, USA) installed in the Armed Forces of Immunology CMH Rawalpindi. To measure serum immunoglobulin levels 2ml blood samples were acquired in a serum separator tube (SST) Address Company. Lymphocyte subset analysis and serum Immunoglobulin levels were measured in the Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan.

Genetic Testing

A whole blood sample of 3ml was collected (K-EDTA tube) from the patient to perform Whole Exome Sequencing (WES). DNA was extracted from the patient, available parents, and healthy siblings peripheral blood samples using GenEluteTM blood genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA). DNA quantification was accomplished using a Nanodrop1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA). To find a pathogenic variant in SCID DNA of patients (III-2 & III-3) and her mother (II-4) were selected for whole exome sequencing WES). DNA Sanger sequencing was performed to validate the segregation of the mega deletion in available family members III-2, II4 along with controls. The exon-specific primers were created by using PRIMER-3 software (http://bioinfo.ut.ee/primer3-0.4.0/). To perform agarose electrophoresis, Polymerase Chain Reaction (PCR) was performed following the research protocol as discussed in our most recent article [12].

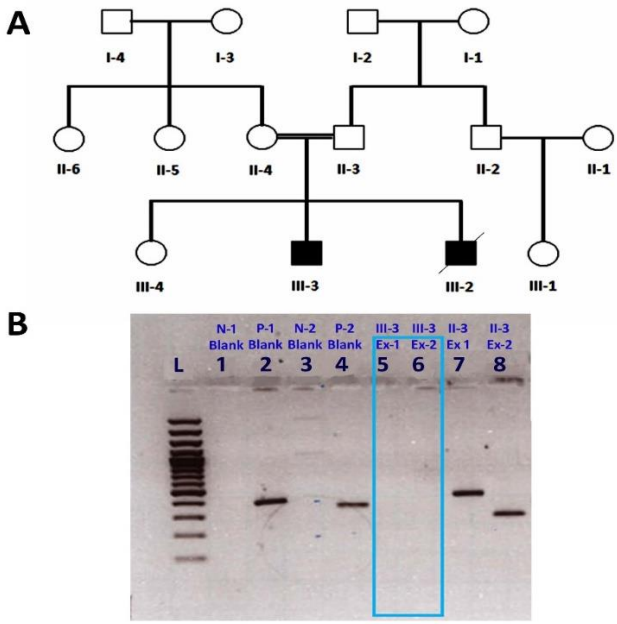
Agarose Gel electrophoresis

The *DCLRE1C* gene amplified PCR products using exon-specific primers were resolved on 2% agarose gel. The 2% Agarose gel (50 volume) was prepared by dissolving 1 gram agarose (Axygen™ Agarose LE USA) in 50 ml 1X Tris-Borate-EDTA (TBE) buffer in a conical flask. The mixture was melted in a microwave oven. Ethidium bromide (Sigma-Aldrich, St. Louis, MO, USA) a DNA intercalating agent was added (0.5 µg/ml final concentration) (). To determine the size of the amplified products 5ul, blank primers and negative blanks and 100 bp DNA ladder (MBI, Fermentas, Life Sciences, UK) were used. The electrophoresis was performed using 80mA for approximately 30-40 minutes in a horizontal gel electrophoresis apparatus (Bio-Rad, USA) in a TE buffer. After the complete electrophoresis run for 30 – 40 minutes, the agarose gel was placed on a UV transilluminator (Biometra®, Göettingen, Germany) to visualize amplified PCR products as shown in (Figure 1B).

Figure 1A: Pedigree of SCID Family: Showing three-generation family segregating SCID. Blank circles and squares represent healthy while shaded circles and squares present affected male and female patients, respectively. Double lines indicate cousin marriages. The diagonal line on III-2 shows the deceased patient.

Figure 1B: Agarose Gel Electrophoresis: "L" represents the ladder. 1 and 3 are Primer 1 and Primer 3 blanks while 2 and 4 showing Primer 1 and Primer 2 only

(without amplified PCR product). 5 and 6 show PCR amplified product of exon 1 and 2 (PCR Failed to amplify due to deleted exons. 7 and 8 showing exon 1 and 2 amplified product in control.



3. Results

Patient Clinical Findings:

In the current research project, we enrolled a 3-month-old male patient. According to the patient's mother, family history was positive with one male patient suffering from similar disease symptoms and dying at the age of 6 months. Parents were first cousins and the pedigree analysis is evidence of a recessive mode of inheritance. The patient was suffering from fever (100°F) with a heartbeat of 88 b/m, lower respiratory tract infections, watery diarrhoea, and oral moniliasis from the age of one month. He was born at normal term with assisted delivery with a weight of 2.5 kilograms. No facial dysmorphism or neurological or physical abnormality was observed. He was diagnosed as a case of severe combined immunodeficiency (SCID) based on criteria issued by the International Union of Immunological Societies (IUIS).

Lymphocyte Subset Analysis)

Lymphocyte subset analysis showed B-lymphocyte and T-lymphocyte negative (B^-/T^-) type of SCID with a total absence of CD3 cells, CD3+CD4 cells, CD3&CD8 cells, CD19 cells and CD4:CD8 ratio was zero. Total TLC was 4900/µl, lymphocyte percentage was 16% and lymphocyte count was 784/µl. The NK cell markers including CD16 and CD56 were normal (78% = 612). All the T-lymphocyte, B-lymphocyte and NK cell concentrations are shown in (Table 1). These findings are suggestive of primary immune deficiency. Serum IgG, IgM, and IgA were diminished. As a control, we measured immunoglobulin levels in the mother. The detailed patients (P1 and P2) and control (mother) results are shown in (Table 1). While the Nitroblue tetrazolium (NBT) test was normal.

Table 1: Lymphocyte Subset Analysis & Serum Immunoglobulin Levels

S. No	Parameters	P (III-3)	P (III-2)	Control (II-4)	Reference Range 1 day – 11 months
	Peripheral blood lymphocyte subset analysis				
1.	TLC	4300/µl	3900/µl	9200/µl	6400-11000/µl
2.	Lymphocyte Percentage	16%	14%	45%(2900)	38-59%
3.	Lymphocyte Count	784/µl	680/µl	50%(4600)	2700-5400
4.	$CD3^+$ cells	0% (0)	0%(0)	75% (1975)	58-67%(1700-3600)
5.	$CD3^+CD4^+$cells	0% (0)	0%(0)	65% (932)	38-50%(1700-2800)
6.	$CD3^+CD8^+$cells	0.1 % (0)	0%(0)	40% (998)	18-25%(800-1200)
7.	$CD19^+$ cells	0.1 % (0)	0%(0)	40% (1085)	19-31%(500-1500)
8.	$CD19^+CD56^+$cells	78% (612)	74% (595)	27% (580)	8-17%(300-700)
9.	CD4:CD8	0.0	0.0	3.90	1.5-2.9
	Immunoglobulin Levels				
10.	IgG	3.4			670–1530 mg/dl
11.	IgA	0.6			52–274mg/dl
12.	IgM	1.6			48–179mg/dl

Genetic Analysis

A detailed whole exome analysis does not yield any pathogenic variant. Copy number variation (CNV) repeats revealed a mega deletion on chromosome 10p13 harbouring the DCLRE1C gene. To find deletion all the DCLRE1C gene coding exons were PCR amplified. PCR amplified products of DCLRE1C exons were run on a 2 percent Agarose gel. The gel electrophoresis revealed exon 1 and 2 deletions (Figure 1B). Parents as well as control samples were successfully amplified and showed exon-specific bands.

4. Discussion

The gene DCLRE1C is destined to encode Artemis, an endonuclease protein that is considered to be crucial for V(D)J recombination and the non-homologous end-joining (NHEJ) pathway, belonging to the Metallo-β lactamase superfamily [13]. The Variable (V), Diversity (D), and Joining (J) chain recombination called V(D)J recombination is a prerequisite for the production of early development of T and B lymphocytes [14]. Mutations in DCLRE1C lead to Artemis deficiency and most of the patients with Artemisdeficiency have SCID and cellular radiosensitivity [15].

In the present research, we report a male Pakistani patient with failure to thrive and recurrent infections including respiratory tract infections (pneumonia) and chronic diarrhoea. The patient's clinical manifestations including pneumonia, diarrhea, and skin rashes were similar to those reported earlier [5,8,10]. Lymphocyte analysis revealed a $T^-/B^-/NK^+$ type of SCID these lab reports are also coherent with those reported earlier (Table 1) [6,7,8]. Artemis is an endonuclease that plays a critical role in V(D)J recombination along with RAG1/2 genes, leading to hypoimmunoglobulenemia (low serum immunoglobulin levels). The patient reported here also showed diminished serum immunoglobulin levels (IgG, IgA, IgM) levels (Table 1) which is also reported in earlier reports [5,8,10].

The gene DNA Cross-Link Repair 1C (DCLRE1C) comprises 12 coding exons which encode to yield a 692 amino acid protein Artemis, weighing 78436 Da. This protein interacts with ATM and BRCA1 protein to produce a quaternary structure[3].

Whole exome sequencing revealed no missense or nonsense mutation in reported SCID-causing genes. Detailed CNV analysis revealed a mega deletion in a region harbouring the DCLRE1C gene. Electrophoresis of exon-specific amplified PCR revealed two exons (exon 1 and 2) deletion (Figure 1B) [6, 15]. This is the first time we are reporting the first two exon mutations in the Pakistani population in the DCLRE1C gene. Earlier in two different patients' exon (1-3) and exon (1-4) deletion was reported in the DCLRE1C gene in the Iranian population [8]. The first two exons deletion in our patient resulted in the deletion of the first 54 amino acids. Artemis belongs to a superfamily of nucleases containing metallo-β-lactamase (MBL) and β-CASP (CPSF-Artemis-SNM1-Pso2) domains [13]. Theβ-lactamase domain encoded by the first four exons of the DCLRE1C gene which harbors a catalytic activity, is a conserved domain involved in hairpin DNA opening activity[16]. Therefore, it is predicted that the two exon deletions identified in this report severely affect the function of Artemis and cause SCID in our patient.

5. Conclusion

The current study narrates clinical and genetic investigation in a patient suffering from Artemis deficiency with B/T cell deficiency. Copy Number Variation (CNV) analysis revealed a novel exon two-exon (1 and 2) deletion in the gene *DCLRE1C*. The detailed clinical and laboratory findings discussed will help physicians in making proper diagnoses of patients with similar disease manifestations.

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S.A, A.Z, N.S, Z.K.K, L.S, S.I.R - Analysis/Interpretation/Discussion
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References

1. Ashby KM, Hogquist KA. A guide to thymic selection of T cells. Nature Reviews Immunology. 2024 Feb;24(2):103-17.https://doi.org/10.1038/s41577-023-00911-8
2. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma & Clinical Immunology. 2018 Sep;14:1-0.https://doi.org/10.1186/s13223-018-0278-1
3. Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, Huang J, Yu Y, Fan XG, Yan Z, Sun X. HMGB1 in health and disease. Molecular aspects of medicine. 2014 Dec 1;40:1-16. https://doi.org/10.1016/j.mam.2014.05.001
4. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, Franco JL, Holland SM, Klein C, Morio T, Ochs HD. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. Journal of Clinical Immunology. 2020;40:24-64. https://doi.org/10.1007/s10875-019-00737-x
5. Meric Z, Gemici Karaaslan B, Yalcin Gungoren E, Bektas Hortoglu M, Cavas T, Aydemir S, Bilgic Eltan S, Firtina S, Kendir Demirkol Y, Eser M, Cekic S. Artemis deficiency: A large cohort including a novel variant with increased radiosensitivity. Pediatric allergy and immunology. 2024; 35(6):e14171.https://doi.org/10.1111/pai.14171
6. Volk T, Pannicke U, Reisli I, Bulashevska A, Ritter J, Björkman A, Schäffer AA, Fliegauf M, Sayar EH, Salzer U, Fisch P. DCLRE1C (ARTEMIS) mutations causing phenotypes ranging from atypical severe combined immunodeficiency to mere antibody deficiency. Human molecular genetics. 2015 Dec 20;24(25):7361-72.https://doi.org/10.1093/hmg/ddv437
7. Strubbe S, De Bruyne M, Pannicke U, Beyls E, Vandekerckhove B, Leclercq G, De Baere E, Bordon V, Vral A, Schwarz K, Haerynck F. A novel non-coding variant in DCLRE1C results in deregulated splicing and induces SCID through the generation of a truncated ARTEMIS protein that fails to support V (D) J recombination and DNA damage repair. Frontiers in Immunology. 2021;17;12:674226. https://doi.org/10.3389/fimmu.2021.674226
8. Ghadimi S, Jamee M, Abolhassani H, Parvaneh N, Rezaei N, Delavari S, Sadeghi-Shabestari M, Tabatabaei SR, Fahimzad A, Armin S, Chavoshzadeh Z. Demographic, clinical, immunological, and molecular features of iranian national cohort of patients with defect in DCLRE1C gene. Allergy, Asthma & clinical immunology. 2023;19(1):13. https://doi.org/10.1186/s13223-023-00768-5
9. Abolhassani H, Kiaee F, Tavakol M, Chavoshzadeh Z, Mahdaviani SA, Momen T, Yazdani R, Azizi G, Habibi S, Gharagozlou M, Movahedi M. Fourth update on the Iranian National Registry of Primary Immunodeficiencies: integration of molecular diagnosis. Journal ofclinical immunology. 2018;38:816-832. https://doi.org/10.1007/s10875-018-0556-1
10. Fayyaz H, Zaman A, Shabbir S, Khan ZK, Haider N, Saleem AF, Ahamad W, Ullah I. Mutational analysis in different genes underlying severe combined immunodeficiency in seven consanguineous Pakistani families. Molecular biology reports. 2024;51(1):302. https://doi.org/10.1007/s11033-024-09222-0.
11. Awais S, Siddique L, Shafi N, Humerah S, Niazi S, Omer W. Genetic Analysis Reveals A Missense Mutation In Reactivation Gene-2 Causing Severe Combined Immunodeficiency Disease In A Pakistani Family: Missense mutation in RAG2 gene causing SCID. Journal of Rawalpindi medical college. 2024 29;28(2). https://doi.org/10.37939/jrmc.v28i2.2588
Nasir r, shabbir s, siddique l, awais s, khalid z, zahoor a, azeem z, majeed f. Integrin beta2 gene variant causing leukocyte adhesion deficiency type 1 in a pakistani family. Pakistan journal of physiology. 2024;20(1):15-18. https://doi.org/10.69656/pjp.v20i1.1614
12. Yosaatmadja Y, Baddock HT, Newman JA, Bielinski M, Gavard AE, Mukhopadhyay SM, Dannerfjord AA, Schofield CJ, McHugh PJ, Gileadi O. Structural and mechanistic insights into the Artemis endonuclease and strategies for its inhibition. Nucleic cids esearch. 202120;49(16):9310-26. https://doi.org/10.1093/nar/gkab693
13. Niewolik D, Schwarz K. Physical ARTEMIS:DNA-PKcs interaction is necessary for V(D)J recombination. Nucleic acids research. 2022;50(4):2096-2110. https://doi.org/10.1093/nar/gkac071
14. Keleş S, Sayar EH, Guner S, Resili İ, Karaselek MA, Kapakli H. Identification of the Mutation in DCLRE1C Gene by PCR-RFLP. Selçuk Tıp Dergisi. 2023;39(1);24-28. https://doi.org/10.1093/cei/uxac041.
15. Gratia M, Rodero MP, Conrad C, Bou Samra E, Maurin M, Rice GI, Duffy D, Revy P, Petit F, Dale RC, Crow YJ. Bloom syndrome protein restrains innate immune sensing of micronuclei by cGAS. Journal of Experimental Medicine. 2019 May 6;216(5):1199-213. https://doi.org/10.1084/jem.20181329