Case Report

Homozygous Autosomal Recessive DIAPH1 Mutation Associated with Central Nervous System Involvement and Aspergillosis: A Rare Case

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The DIAPH1 gene fulfills critical immune and neurodevelopmental roles. It encodes the mammalian Diaphanous-related formin (mDia1) protein, which acts downstream of Rho GTPases to promote F-actin polymerization and stabilize microtubules. During mitosis, this protein is expressed in human neuronal precursor cells and considerably affects spindle formation and cell division. In humans, dominant gain-of-function DIAPH1 variants cause sensorineural deafness and macrothrombocytopenia (DFNA1), while homozygous DIAPH1 loss leads to seizures, cortical blindness, and microcephaly syndrome (SCBMS). To date, only 16 patients with SCBMS have been reported, none of whom were from Iran. Furthermore, aspergillosis is yet to be reported in patients with homozygous DIAPH1 loss, and the link between SCBMS and immunodeficiency remains elusive. In this study, we shed further light on this matter by reporting the clinical, genetic, and phenotypic characteristics of an Iranian boy with a long history of recurrent infections, diagnosed with SCBMS and immunodeficiency (NM_005219.5 c.3145C>T; p.R1049X variant) following aspergillosis and SARS-CoV-2 coinfection.

1. Introduction

The DIAPH1 gene fulfills critical immune and neurodevelopmental roles; it encodes the mammalian Diaphanous-related formin (mDia1) protein, which acts downstream of Rho GTPases to promote F-actin polymerization and stabilize microtubules [1–3]. Besides its role in malignancies, the DIAPH1 gene has been implicated in various Mendelian diseases [4, 5]. Dominant gain-of-function DIAPH1 variants cause sensorineural deafness and macrothrombocytopenia (DFNA1) [6–8], while homozygous DIAPH1 loss leads to seizures, cortical blindness, and microcephaly syndrome (SCBMS). In this syndrome, seizures usually develop during early infancy and are challenging to manage. Though body measurements may be normal at birth, microcephaly, cortical blindness, and intellectual disability gradually develop, sometimes with stunted growth [9–11].

To date, only 16 patients with SCBMS have been reported, including the following variants: NM_005219: c.2332C>T; p. Q778X, c.2769delT; p.F923fs, c.3145C>T; p.R1049X; and c.68411G>A [9–11]. Only one of the previous studies highlights a clear link between SCBMS and immunodeficiency [11], though some cases with bronchiectasis or recurrent respiratory infections have also been described [9, 10]. Furthermore, aspergillosis is yet to be reported in patients with homozygous DIAPH1 loss. Herein, we shed further light on this matter by reporting the case of an Iranian boy with a long history of recurrent infections, diagnosed with SCBMS and immunodeficiency (NM_005219.5 c.3145C>T; p.R1049X variant) following aspergillosis and SARS-CoV-2 coinfection.
2. Case Presentation

In February 2022, we admitted a four-year-old boy, who was a known case of CD4 deficiency, seizure, cortical blindness, and microcephaly, due to fever. On physical examination, the vital signs were stable, with the exception of a 38.7°C temperature. The skin featured a scar from a past vasculitis lesion, and both eyes had no response to light. Upon auscultation of the lungs, bilateral rales were detected. Upon admission, his weight, height, and head circumference were 12 kg (z-score: −3.37), 59 cm (z-score: −10.32), and 44 cm, respectively.

The patient had a history of seizures, blindness, and recurrent infections since birth. His birth weight was 2.98 kg, his height was 49 cm, and his head circumference was 34 cm, and the parents were distantly related. Neonatal screening for inborn errors of metabolism and endocrinopathies was normal. His seizures had commenced during early infancy in generalized tonic-clonic form; the patient was on antiseizure medications for a period of time, after which the seizures no longer occurred and the medications were tapered and discontinued. Before admission, the patient was receiving prophylactic cotrimoxazole and fluconazole. He had no family history of immunodeficiency or death of unknown cause. He had previously been hospitalized many times, and a summary of the previous five admissions is presented in Table 1. In terms of surgical history, the patient underwent a right posterior lateral thoracotomy and right upper lobectomy when he was one and a half years old due to congenital lobar emphysema. Due to the known immunodeficiency status, broad-spectrum antibiotics (intravenous vancomycin and meropenem) were initiated upon admission.

In the laboratory workup, the following findings were significant: WBC 13,700/μl (62% lymphocytes), Hb 11.1 g/dl, Plt 475,000/μl, ESR 65 mm/hr, CRP 38 mg/l, D-dimer 4,360 ng/ml (high), ferritin 506.4 ng/ml (high), procalcitonin 0.69 ng/ml (equivocal), SARS-CoV-2 IgG 0.2 (negative), SARS-CoV-2 IgM <0.01 (negative), negative serum aspergillus galactomannan Ag, and normal total IgE and HIV Ab/IgM <0.01 (negative), negative serum aspergillus galactomannan Ag, and normal total IgE and HIV Ab/IgM <0.01 (negative). Notably, he had the same variant (c.3145C>T; p.R1049X) variant. In that report, immunodeficiency was not mentioned, though one patient succumbed to a chest infection at the age of 18 [10]. In the second report on this issue, the variants were detected in four individuals with SCBMS from two unrelated consanguineous families. The first was a boy from the United Arab Emirates who had SCBMS as well as recurrent sino-pulmonary infections (causing chronic cough and secretions), though sweat testing and immunological function screenings were normal. He also suffered from wheezing early on and needed supplemental oxygen for seven months. Notably, he had the same variant (c.3145C>T; p.R1049X) as our patient. The other three were Omani siblings (two females; one male) who had the c.2769delT; p.F923fs variant of homozygous DIAPH1 loss. Again, immunodeficiency was not detected, though one female had severe bronchiectasis and died of pneumonia at age 13 [9].

In the only report to have linked homozygous DIAPH1 loss with SCBMS and immunodeficiency, Kaustio et al. described five Finnish patients homozygous for the NM_005219:c.2769delT; p.F923fs variant. In that report, immunodeficiency was not detected, though one female had severe bronchiectasis and died of pneumonia at age 13 [9].

In his study, we present the characteristics of an Iranian boy with SCBMS and CD4 deficiency, reporting the first case of aspergillosis associated with the NM_005219.5 c.3145C > T; p.R1049X variant of homozygous DIAPH1 loss. The first reported instance of such a mutation affected five siblings in a Saudi Arabian family, who had developmental delay, intellectual disability, blindness, microcephaly, seizures, and stunted growth caused by the c.2332C>T variant (p.Gln778R, RefSeq NM_005219.4) variant. In that report, immunodeficiency was not mentioned, though one patient succumbed to a chest infection at the age of 18 [10]. In the second report on this issue, the variants were detected in four individuals with SCBMS from two unrelated consanguineous families. The first was a boy from the United Arab Emirates who had SCBMS as well as recurrent sino-pulmonary infections (causing chronic cough and secretions), though sweat testing and immunological function screenings were normal. He also suffered from wheezing early on and needed supplemental oxygen for seven months. Notably, he had the same variant (c.3145C > T; p.R1049X) as our patient. The other three were Omani siblings (two females; one male) who had the c.2769delT; p.F923fs variant of homozygous DIAPH1 loss. Again, immunodeficiency was not detected, though one female had severe bronchiectasis and died of pneumonia at age 13 [9].

Located on chromosome 5 in humans, the DIAPH1 gene fulfills a crucial role in brain development [10]. This gene is responsible for encoding the RhoA GTPase mDia1 [12, 13], which binds to F-actin to stimulate the addition of actin monomers following activation by RhoA [14]. During mitosis, this protein is expressed in human neuronal precursor cells and considerably affects spindle formation and cell division [10]. Pathogenic variants of the DIAPH1 gene are involved in a number of diseases: heterozygous gain-of-function variants cause deafness and thrombocytopenia (OMIM 124900) [6, 7] and have also been associated with sporadic moyamoya disease [15]. In contrast, homozygous loss-of-function variants result in SCBMS (OMIM 616632) [9–11].
Table 1: Summary of the previous five hospital admissions of the patient.

| Admission (years and months) | First | Second | Third | Fourth | Fifth |
|------------------------------|-------|--------|-------|--------|-------|
| Age                          | 1 y 4 m | 1 y 6 m | 2 y 5 m | 2 y 7 m | 3 y 7 m |
| Chief complaint              | Productive cough, vomiting | Cough | Fever, cough | Fever | Fever |
| Diagnosis                    | Pneumonia | Congenital lobar emphysema | Sepsis vs. pneumonia, CD4 deficiency | COVID-19, sepsis | Suspected sepsis |
| Duration of admission (days) | 7 | 5 | 10 | 14 | 5 |

Significant laboratory data

- (i) WBC 11,100/μl
- (ii) Hb 11.6 g/dl
- (iii) Plt 285,000/μl
- (iv) Brain MRI: Thin corpus callosum splenium suggestive of mid-central cortical atrophy
- (v) Spiral chest CT: Multiple cystic structures measuring about 60x55x 40 mm in mid-zone of right lung contains multiple air-containing cysts with variable size and septation suggestive of type 1 congenital pulmonary airway malformation. Diffuse patchy air space opacities in both upper and lower lobes with multiple air space nodules suggestive of bronchopneumonia
- (vi) Bone marrow aspiration biopsy/immunohistochemistry: erythroid hypoplasia with parvovirus B19 infection; 4-5% immature myeloid cells; about 30% hematogones
- (vii) Left leg lesion skin biopsy: lymphocytic vasculitis
- (viii) ANA 3.9 U/ml†
- (ix) dsDNA (IgG) 26.01 IU/ml†
- (x) dsDNA (IgM) 32.71 IU/ml†
- (xi) ACLA IgM 51.1 IU/ml†
- (xii) Anti RO (SSA) IU/ml 83.2†
- (xiii) IgG 16.34 g/l†
- (xiv) IgM 1.97 g/l†
- (xv) IgA 1.21 mg/dl†
- (xvi) IgE 15.8 IU/ml (normal)
- (xvii) ASMA neg.
- (xviii) P-ANCA (Anti MPO) neg.
- (xix) C-ANCA (Anti PR3) 132.0 IU/ml†

- (i) WBC 3000/μl (32% lymph.)
- (ii) Hb 6.6 g/dl
- (iii) Plt 488,000/μl
- (iv) Flow cytometry: CD3 71%, CD4 12%, CD8 55%†, CD16 23%, CD19 6%, CD56 23%, CD14 16%, CD4/CD8 0.22†
- (v) Karyotype study on bone marrow culture: normal (46,XY)
- (vi) Bone marrow aspiration biopsy/immunohistochemistry: erythroid hypoplasia with parvovirus B19 infection; 4-5% immature myeloid cells; about 30% hematogones
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† High/positive; ‡ Borderline/equivocal; § Low.
infection, though he also developed COVID-19 and aspergillosis, which were yet to be reported in patients with homozygous DIAPH1 loss. Aspergillosis is an opportunistic fungal infection that can be life-threatening in those who are immunocompromised. Factors that render an individual susceptible include neutropenia, corticosteroid use, hematologic malignancies, diabetes, underlying lung disease, and AIDS [16–19], with low CD4 counts being associated with a higher incidence of this condition [20]. In our patient, hematologic malignancies were ruled out and corticosteroids were not routinely used, though underlying lung disease was present in addition to a low CD4 count (920/\mu l).

According to the Kaustio et al. study, patients with SCBMS can have combined immune deficiency, as autosomal skeletal organization disorganization and mitochondrial dysfunction are implicated in the pathogenesis of the syndrome [11]. In that study, T cells derived from the patients had impaired adhesion and inefficient microtubule-organizing center translocation to the immune synapse, which is essential for T-cell function. Such a defect has previously been shown in animal models, so immunodeficiency can be expected in such patients given the functions of DIAPH1 in actin nucleation and microtubule organization and considering that mutations in several cytoskeleton-regulating genes cause primary immunodeficiency [11, 21–23].

An incidental finding reported in our genetic workup was the heterozygous c.1129 C>T variant in the DNAJC3 gene. While homozygous mutations in DNAJC3 on chromosome 13q32 cause combined cerebellar and peripheral ataxia with hearing loss and diabetes mellitus (ACPHD) [24], there is no evidence to suggest a link between the heterozygous variant found incidentally in our patient and his specific phenotype. Rather, the phenotypic features seen in our case are generally well-aligned with the prior reports on homozygous DIAPH1 loss, indicating that this DIAPH1 variant was responsible for the observed phenotype.

The strengths of this study include the characterization of the phenotype related to a very rare form of immunodeficiency for the first time in an Iranian patient. The main limitation is the cross-sectional nature of this report, though the patient remains under our follow-up and longitudinal data can be collected in the future. Our results add evidence in support of the link between homozygous DIAPH1 loss and T-cell deficiency. Hence, physicians who manage SCBMS patients must be prepared to prevent or treat severe or recurrent infections, and corticosteroid use may need to be limited in these patients to prevent aspergillosis. Further investigations should be conducted into the exact mechanism behind the defective T-cell responses in patients with homozygous DIAPH1 loss.

### Ethical Approval

A written informed consent was obtained from the patient’s guardian for performing and publishing this study.

### Conflicts of Interest

The authors declare that there are no conflicts of interest.

### References

[1] F. Bartolini and G. Gundersen, “Formins and microtubules,” *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1803, no. 2, pp. 164–173, 2010.

[2] A. Palazzo, B. Ackerman, and G. G. Gundersen, “Tubulin acetylation and cell motility,” *Nature*, vol. 421, no. 6920, p. 230, 2003.

[3] T. Tominaga, E. Sahai, F. McCormick, S. A. Courtneidge, and A. S. Alberts, “Diaphanous-related formins bridge Rho GTPase and Src tyrosine kinase signaling,” *Molecular Cell*, vol. 5, no. 1, pp. 13–25, 2000.

[4] K. M. Eisenmann, K. J. Dykema, S. A. Courtneidge, and A. S. Alberts, “Diaphanous-related formins bridge Rho GTPase and Src tyrosine kinase signaling,” *Molecular Cell*, vol. 5, no. 1, pp. 13–25, 2000.

[5] S. Narumiya, M. Tanji, and T. Ishizaki, “Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion,” *Cancer and Metastasis Reviews*, vol. 28, pp. 65–76, 2009.

[6] E. D. Lynch, M. K. Lee, J. E. Morrow, P. L. Welch, P. E. León, and M.-C. King, “Nonsyndromic deafness DFNA1 associated
with mutation of a human homolog of the Drosophila gene diaphanous,” Science, vol. 278, no. 5341, pp. 1315–1318, 1997.
[7] S. Stritt, P. Nurden, E. Turro et al., “A gain-of-function variant in DIA1 causes dominant macrothrombocytopenia and hearing loss,” Blood, vol. 127, no. 23, pp. 2903–2914, 2016.
[8] T. Ueyama, Y. Ninoyu, S. Y. Nishio et al., “Constitutive activation of DIA1 (DIAH1) via C-terminal truncation causes human sensorineural hearing loss,” EMBO Molecular Medicine, vol. 8, no. 11, pp. 1310–1324, 2016.
[9] A. Al-Maawali, B. J. Barry, A. Rajab et al., “Novel loss-of-function variants in DIAH1 associated with syndromic microcephaly, blindness, and early onset seizures,” American Journal of Medical Genetics, Part A, vol. 170, no. 2, pp. 435–440, 2016.
[10] A. G. Ercan-Sencicek, S. Jambi, D. Franjic et al., “Homozygous loss of DIAH1 is a novel cause of microcephaly in humans,” European Journal of Human Genetics, vol. 23, no. 2, pp. 165–172, 2015.
[11] M. Kaustio, N. Nayebzadeh, R. Hinttala et al., “Loss of DIA1 causes SCBMS, combined immunodeficiency, and mitochondrial dysfunction,” The Journal of Allergy and Clinical Immunology, vol. 148, no. 2, pp. 599–611, 2021.
[12] A. Shimada, M. Nyitrai, I. R. Vetter et al., “The core FH2 domain of diaphanous-related formins is an elongated actin binding protein that inhibits polymerization,” Molecular Cell, vol. 13, no. 4, pp. 511–522, 2004.
[13] R. Shinohara, D. Thumkeo, H. Kamiyo et al., “A role for mDia, a Rho-regulated actin nucleator, in tangential migration of interneuron precursors,” Nature Neuroscience, vol. 15, no. 3, pp. 373–380, 2012.
[14] B. L. Goode and M. J. Eck, “Mechanism and function of formins in the control of actin assembly,” Annual Review of Biochemistry, vol. 76, no. 1, pp. 593–627, 2007.
[15] A. J. Kundishora, S. T. Peters, A. Pinard et al., “DIAH1 variants in non–east asian patients with sporadic moyamoya disease,” JAMA Neurology, vol. 78, no. 8, pp. 993–1003, 2021.
[16] D. W. Denning, “Invasive aspergillosis,” Clinical Infectious Diseases, vol. 26, no. 4, pp. 781–803, 1998.
[17] D. W. Denning, S. E. Follansbee, M. Scolaro, S. Norris, H. Edelstein, and D. A. Stevens, “Pulmonary aspergillosis in the acquired immunodeficiency syndrome,” New England Journal of Medicine, vol. 324, no. 10, pp. 654–662, 1991.
[18] O. Lortholary, M.-C. Meyohas, B. Dupont et al., “Invasive aspergillosis in patients with acquired immunodeficiency syndrome: report of 33 cases,” The American Journal of Medicine, vol. 95, no. 2, pp. 177–187, 1993.
[19] N. Singh, V. L. Yu, and J. D. Rihs, “Invasive aspergillosis in AIDS,” Southern Medical Journal, vol. 84, no. 7, pp. 822–827, 1991.
[20] K. J. Holding, M. S. Dworkin, P.-C. T. Wan et al., “Aspergillosis among people infected with human immunodeficiency virus: incidence and survival,” Clinical Infectious Diseases, vol. 31, no. 5, pp. 1253–1257, 2000.
[21] T. S. Gomez, K. Kumar, R. B. Medeiros, Y. Shimizu, P. J. Leibson, and D. D. Billadeau, “Formins regulate the actin-related protein 2/3 complex-independent polarization of the centrosome to the immunological synapse,” Immunity, vol. 26, no. 2, pp. 177–190, 2007.
[22] E. Janssen and R. S. Geha, “Primary immunodeficiencies caused by mutations in actin regulatory proteins,” Immunological Reviews, vol. 287, no. 1, pp. 121–134, 2019.
[23] M. Kloc, J. Z. Kubiak, X. C. Li, and R. M. Ghobrial, “The newly found functions of MTOC in immunological response,” Journal of Leukocyte Biology, vol. 95, no. 3, pp. 417–430, 2014.