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Brief Communication

DNMT3B deficiency presenting as severe combined immune deficiency: A case report

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A B S T R A C T

Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome is a group of rare autosomal recessive disorders. The immune disease in the ICF syndrome consists mainly of humoral immunodeficiency. T-cell dysfunction has previously been suspected to be part of the syndrome's spectrum. However, patients with ICF display, at a young age, a normal number of T cells that tend to decline throughout disease progression due to apoptosis. Biallelic mutations in the *DNMT3B* gene account for around 50% of ICF cases (ICF type 1). The remaining half may be linked to *ZBTB24*, *CDCA7* or *HELLS*. Here we report a novel homozygous *DNMT3B* mutation (NM_ 006892; p.R826H) in a Lebanese family presenting in early infancy with severe combined immune deficiency (SCID). This work expands the clinical spectrum of the ICF syndrome and confirms the importance of tailoring therapeutic approaches for each patient with ICF syndrome, according to the clinical manifestations of his disease.

1. Introduction

Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome is a group of rare autosomal recessive disorders. First described in 1978 in patients with a variable Primary Immunodeficiency Disease (PID) and centromeric instability of chromosomes 1, 9 and 16 [1,2]; the acronym ICF was then attributed to the disease by Maraschio and colleagues based on the diagnostic triad consisting of variable immunodeficiency, cytogenetic abnormalities and facial dysmorphism [3]. Other manifestations of ICF may include failure to thrive, growth retardation, neurological defects (neurodevelopmental delay, intellectual disability, psychomotor delay, *etc.*) [4–8] and hematological malignancies [6,8,9]. Patients with ICF syndrome have mild facial dysmorphism including hypertelorism, flat nasal bridge and epicanthic folds, in addition to other features such as macroglossia, telecanthus, micrognathia and protrusion of the tongue [4,6,8,10]. Characteristic chromosomal aberrations in ICF syndrome are multiradial chromosome junctions, whole-arm deletions, chromatid breaks and decondensation of the juxtacentromeric heterochromatin region (qh) predominantly of chromosomes 1, 16 and sometimes 9, in mitogen-stimulated lymphocytes [11–13]. Patients with ICF syndrome suffer mainly from severe recurrent respiratory tract and gastrointestinal infections associated with humoral immunodeficiency [8]. They present with agammaglobulinemia or hypogammaglobulinemia in the presence of normal numbers of circulating B cells [4,10]. However, their B cell repertoire contains an increased proportion of immature naïve cells and lacks memory cells [7,8]. In addition, some patients with ICF syndrome present with opportunistic infections including *Pneumocystis jiroveci* and persistent fungal infections [6,8,10,11,14], suggesting a T-cell dysfunction. Biallelic mutations in the *DNMT3B* gene (#MIM 602900), encoding the DNA methyltransferase 3B, are associated with ICF type 1 (ICF1, MIM#242860) [13,15], which accounts for around 50% of all ICF cases [6,8,9]. The remaining half may be caused by mutations in *ZBTB24* (ICF2, MIM#614069) [16], *CDCA7* (ICF3, MIM#616910) or *HELLS* (ICF4, MIM#616911) [8,17]. DNMT3B belongs to the DNMT3 family of DNA methyltransferases (DNMTs) that modulate *de novo* DNA methylation patterns during embryogenesis and early development [18]. It is solely responsible for *de novo* methylation

Abbreviations: DNMTs, DNA Methyltransferases; HSCT, Hematopoietic Stem Cell Transplantation; ICF, Immunodeficiency, Centromeric Instability, and Facial Anomaly Syndrome; PID, Primary Immunodeficiency Disease; SCID, Severe Combined Immunodeficiency Disease; WES, Whole Exome Sequencing

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of centromeric minor satellite repeats [19] whereas ZBTB24, CDCA7, and HELLS contribute to the DNMT1-dependent DNA methylation maintenance pathway [20]. DNA hypomethylation of pericentromeric satellite repeats, with mutations in any of these four genes, is the molecular hallmark of the ICF syndrome [20]. Immunoglobulin replacement therapy is considered to be the standard treatment for patients with ICF [4,6], while hematopoietic stem cell transplantation (HSCT) may be required to cure the severe forms of the disease [8,9,11]. We herein report a novel homozygous mutation in the *DNMT3B* gene in a Lebanese family presenting with ICF type 1 and severe T-cell lymphopenia at birth. We also review T-cell defects in reported patients with ICF syndrome.

2. Material and methods

a) Study subjects

Written informed consent for genetic analysis and data publication was obtained from the family in compliance with national ethics regulation.

b) Whole Exome Sequencing (WES):

Genomic DNA was isolated from peripheral blood by standard salt-precipitation methods [21]. Exon capture and sequencing: The exome was captured using the SureSelect Human All Exons, reagents (Agilent Inc.® Santa Clara, CA) according to the manufacturer's standard protocol. The concentration of each library was determined using Agilent's QPCR NGS Library Quantification Kit (G4880A). Samples were pooled prior to sequencing with a final concentration of each sample equal to 10 nM. Sequencing was performed on the Illumina HiSeq2000 platform using TruSeq v3 chemistry. Mapping and alignment: Reads files (FASTQ) were generated from the sequencing platform *via* the manufacturer's proprietary software. Reads were aligned to the hg19/b37 reference genome using the Burrows-Wheeler Aligner (BWA) package v0.6.1 [22]. Local realignment of the mapped reads around potential insertion/deletion (Indel) sites was carried out with the Genome Analysis Tool Kit (GATK) [23]. Duplicate reads were marked using Picard v1.62. Additional BAM file manipulations were performed with SAM tools 0.1.18 [24]. Base quality (Phred scale) scores were recalibrated using GATK's covariance recalibration. SNP and Indel variants were called for each sample using the GATK Unified Genotyper [25]. Variants were called using high stringency settings and annotated with VarAFT software 1.61 [26] containing information from dbSNP147 and ExAC (http://exac.broadinstitute.org/).

3. Results

3.1. Case presentation

A 3 month-old Lebanese boy (P1) born to second-degree consanguineous parents presented with persistent diarrhea, fever, pneumonia and failure to thrive. Immunophenotyping showed a complete absence of $CD3^+$ T cells, normal B cell count and a slight decrease in NK cell counts (Table 1). The patient P1 passed away of respiratory failure due to pneumonia at 4 months of age. Based on the clinical and immunological findings, a clinical diagnosis of severe combined immunodeficiency disease (SCID) was highly suspected without any further genetic testing.

Because of the family history of SCID, a female sibling (P2) born later, was tested at one week of age. Phenotyping of her lymphocytes showed a low absolute lymphocyte count, $CD3^+$ T cell lymphopenia with drastic decrease in naïve T cells indicating very poor thymic output and suggesting that the detectable T cells, which are mainly $CD45RA^-$ T cells, are most likely of maternal origin (Table 1). A reevaluation of the patient revealed decreased levels of serum immunoglobulins (IgM, IgA and IgG) that was observed in the presence of a normal number of circulating B cells. It also showed a decline in the number of $CD3^+$ T- and NK cells in addition to an inverted CD4/CD8 ratio (equals to 0.16) (Table 1). Unfortunately, specific antibody response was not assessed in this patient and T cell functional testing was not possible because of the unavailability of further patient samples. Upon physical examination, dysmorphic facial features were noted, namely hypertelorism, epicanthic folds, micrognathia and low set ears. Patient P2 is currently 8 months of age. She is receiving intravenous immunoglobulin replacement monthly and is maintained on TMP/SMX, acyclovir and fluconazole prophylaxis. She is doing well and is being considered for haploidentical HSCT.

3.2. Genetic findings

In order to detect the molecular basis of the immunodeficiency disease in the family, WES was conducted for patient P2. Analysis of the 17 genes involved in SCID was first carried out and found to be normal (list of genes is available upon request). All SCID genes were fully covered (at a minimum of $20\times$) by WES. Reanalysis of raw data for a panel of 350 genes involved in PIDs was subsequently performed and revealed the presence of a homozygous missense variant in the *DNMT3B* gene (NM_ 006892; c.2477G > A, p.R826H). The p.R826H variant is located in a hotspot mutational site of the gene, encoding the catalytic domain of the protein. It is predicted to be pathogenic by PolyPhen-2 score of 1, Sorting Intolerant From Tolerant (SIFT) score of 0, and Mutation Taster score of 1; with a Combined Annotation Dependent Depletion (CADD) score of 35. It is also classified as likely pathogenic in accordance with the American College of Medical Genetics guidelines (PM1, PM2, PP2, PP3). Indeed, a pathogenic variant within the same amino acid residue “826” of *DNMT3B* (p.R826C) has previously been reported in a Jordanian patient with ICF type 1 (Hagleitner MM et al., 2008).

4. Discussion

Here we report a novel homozygous mutation in the *DNMT3*B gene in a Lebanese family presenting with SCID in early infancy. Immune workup showed a low absolute lymphocyte count and $CD3^+$ T cell lymphopenia manifesting at birth in two siblings in the family herein reported. Further evaluation of one of the siblings (P2) revealed a drastic decrease in naïve T cells, indicating a poor thymic output and suggesting maternal T cells engraftment in this patient. While the survival of transplacentally acquired maternal T lymphocytes is limited in immunocompetent infants, long-term engraftment of maternal T cells is a well-known phenomenon in SCID patients [27]. Immune evaluation of patient P2 also showed reduced levels of serum immunoglobulins (IgM, IgA and IgG) occurring in the presence of normal numbers of circulating B cells, and a decline in the number of $CD3^+$ T-cells and NK cells throughout the course of the disease. Clinical examination of P2 indicated facial dysmorphology. However, cytogenetic evaluation was not possible due to the unavailability of further patient samples.

DNMT3B acts as a *de novo* methyltransferase. Mutations in *DNMT3B* are associated with ICF syndrome. Patients with this syndrome exhibit instability at the juxtacentromeric regions of chromosomes 1, 16 and 9, facial anomalies in addition to immunodeficiency.

While the immune disease in the ICF syndrome consists mainly of humoral immunodeficiency [4,7,8,10], T-cell dysfunction has previously been suspected to be part of the syndrome's spectrum. Indeed, many patients with ICF syndrome present with opportunistic infections, namely *Pneumocystis jiroveci*, JC virus and *Candida albicans* infections, suggesting a T cell-associated immunodeficiency [6,8,10–12,14]. Reduced *in-vitro* T cell proliferation to mitogenic stimulation has been reported in 7 ICF type 2 [7,8,28] and 5 ICF type 1 patients [6,8]. Individuals affected with ICF display a normal number of T cells at a young age. Though, their T cells, particularly the CD4+ T cells, tend to

Table 1
Immunological profile of the patients included in this study.

Patient	P1	P2	
Gender	Boy	Girl	
Patient age at the time of testing	4 Months	1 Week	5 Months
Hemogram (normal range)			
WBCs, 10^3 cells/µL	6.95 (6.7–14.00)	5.7 (7.2–8.00)	ND
Lymphocytes, 10^3 cells/µL	1.32 (3.90–9.00)	1.9 (3.40–7.60)	1.33 (3.90–9.00)
Lymphocyte subsets			
$CD3^+$, 10^3 cells/µL	UD (2.50–5.60)	0.88 (2.50–5.50)	0.366 (2.50–5.60)
$CD3^+CD4^+$, 10^3 cells/µL	UD (1.80–4.00)	0.37 (0.56–1.70)	0.03 (1.80–4.00)
$CD45RA^+CCR7^+$, % $CD4^+$	ND	2 (78.15–90.70)	0.186
$CD45RA^-CCR7^-$, % $CD4^+$	ND	8 (0.05–0.27)	ND
$CD45RA^-CCR7^+$, % $CD4^+$	ND	89.5 (8.94–21.70)	ND
$CD45RA^+CCR7^-$, % $CD4^+$	ND	0.5 (0.01–0.25)	ND
$CD3^+CD8^+$, 10^3 cells/µL	UD (0.59–1.60)	0.24 (0.3–2.7)	ND (0.59–1.60)
$CD45RA^+CCR7^+$, % $CD8^+$	ND	5 (84.66–92.62)	ND
$CD45RA^-CCR7^-$, % $CD8^+$	ND	39.6 (0.06–0.55)	ND
$CD45RA^-CCR7^+$, % $CD8^+$	ND	19.2 (7.23–11.85)	ND
$CD45RA^+CCR7^-$, % $CD8^+$	ND	36.2 (0.05–1.16)	ND
$CD3^+CD4^-CD8^-$,	ND	0.08	ND
$CD19^+$, 10^3 cells/µL	0.832 (0.43–3.00)	0.30 (0.30–2.00)	0.449 (0.43–3.00)
$CD27^-$, % $CD19^+$	ND	95.29	ND
$CD27^+$, % $CD19^+$	ND	4.71	ND
$CD3^-CD56^+$, 10^3 cells/µL	0.107 (0.17–0.83)	0.36 (0.17–1.10)	0.029 (0.17–0.83)
Immunoglobulins			
IgG, mg/dL	ND	ND (293–806)	35 (139–1081)
IgM, mg/dL	ND	<5 (0 – 20)	<5 (40–230)
IgA, mg/dL	ND	2 (1–34)	0.3 (20 – 130)

ND, not done.
UD, undetectable.

decline throughout the course of the disease [6,8,28,29] due to increased apoptosis [28–30]. CD4+ T cell lymphopenia thus develops in many patients with ICF syndrome [6,29]. A modest CD3+ T lymphopenia was also observed in three adult ICF2 patients (23, 28 and 29 years of age) reported by Kamae and colleagues [7]. However, the severe neonatal T-cell lymphopenia observed in our patients remains an unusual feature of the ICF syndrome. Three clinically diagnosed ICF infants with proven centromeric instability died during early infancy due to enterocolitis or pneumonia. Of these, two patients, a Bulgarian and an Algerian, had low numbers of T cells. However cell counts were not reported and confirmatory genetic testing was not conducted [6].

The homozygous missense variant p.R826H in *DNMT3B* detected in the patient P2 is located in a hotspot mutational site in the C-terminal portion of the gene that harbors the majority of ICF1 variants and encodes the catalytic domain of the protein [8]. Hagleitner and Weemaes previously reported a Jordanian patient with a homozygous p.R826C mutation, within the same amino acid residue of the DNMT3B protein [6,8]. That patient was found to have dysmorphic features at birth (with no other symptoms), which instigated genetic investigations and diagnosis at the age of 5 weeks. Noteworthy, the patient showed absence of IgA and IgM, but did not have T-cell lymphopenia. He presented later with acute lymphoblastic leukemia [6,8]. This highlights once again the clinical heterogeneity of the ICF1 syndrome and the lack of clear genotype-phenotype correlation that was illustrated by several studies [6,8].

DNMT3B is a DNA methyltransferase that carries *de novo* methylation of centromeric minor satellite repeats during embryonic development [19]. While a complete loss of function of *DNMT3B* results in embryonic lethality in mice, hypomorphic mutations in this gene resulting in decreased methylation of satellite DNA, are associated with the ICF1 syndrome. Findings in the murine model suggest that *DNMT3B* mutations do not affect the development of T cells, that are normal at birth, however, they alter the survival of these cells in the thymus after birth, by enabling their apoptosis *via* a p53-independent mechanism [31]. The role of epigenetics and chromatin architecture in the regulation of T-cell fate, stability and survival was further supported by several lines of evidences [32,33]. Altered DNA methylation patterns contribute to immune dysregulation and to the pathogenesis of systemic autoimmune diseases, through the modulation of gene expression [34,35]. However, autoimmunity, mainly autoimmune manifestations presenting in the absence of autoreactive antibodies, were only reported in few patients with ICF [6,7,28]. We speculate that the clinical heterogeneity in T-cell defects observed in the ICF syndrome, may be attributed to the possible contribution of several epigenetic modifiers to the pathogenesis of this disease. While the majority of patients with ICF syndrome would benefit from immunoglobulin replacement therapy; the severe form of the disease observed in our patient would require HSCT. Live-attenuated vaccines should also be strictly avoided in this case. This highlights the importance of tailoring therapeutic approaches for patients with ICF syndrome, according to the clinical manifestations of the disease.

Our findings expand the clinical spectrum of the immunodeficiency related to the ICF syndrome by reporting the occurrence of a severe neonatal T-cell lymphopenia in a family with a novel *DNMT3B* mutation. It also stresses on the importance of early screening and diagnosis for patients born to families with a history of primary immunodeficiency and confirms the importance of tailored therapeutic approaches for patients with PID.

Authorship contributions

CM: conceived the study, performed data analyses and interpretation and drafted the manuscript. HK: collected patient data, followed and treated the patient. GD and RHW: contributed to the follow-up of the patient. CF: contributed to study concept. All authors have read and approved the manuscript.

Ethics approval and consents

Approval to conduct the study was obtained from the Ethics

Committee of Saint Joseph University, Beirut, Lebanon (Reference: CEHDF 1262). Parents signed an informed consent for participation and sample collection.

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Declaration of Competing Interest

None.

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