Short Report

A novel deletion in \(ZBTB24\) in a Lebanese family with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2


The immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome is a rare autosomal recessive disease characterized by targeted chromosome breakage, directly related to a genomic methylation defect. It manifests with phenotypic and clinical variability, with the most consistent features being developmental delay, facial anomalies, cytogenetic defects and immunodeficiency with a reduction in serum immunoglobulin levels. From the molecular point of view, ICF syndrome was always divided into ICF type 1 (ICF1) and ICF type 2 (ICF2). Mutations in \(DNMT3B\) gene are responsible for ICF1, while mutations in \(ZBTB24\) have been reported to be responsible for ICF2. In this study, we describe a Lebanese family with three ICF2 affected brothers. Sanger sequencing of the coding sequence of \(ZBTB24\) gene was conducted and revealed a novel deletion: c.396_397delTA (p.His132Glnfs*19), resulting in a loss-of-function of the corresponding protein. \(ZBTB24\) belongs to a large family of transcriptional factors and may be involved in DNA methylation of juxtacentromeric DNA. Detailed molecular and functional studies of the \(ZBTB24\) and \(DNMT3B\) genes are needed to understand the pathophysiology of ICF syndrome.

Conflict of interest
Nothing to declare.

Immunodeficiency, centromeric instability and facial anomalies (ICF; MIM 242860) syndrome is a rare autosomal disease, characterized by immunodeficiency, chromosome instability, and facial anomalies. Immunodeficiency is due to either agammaglobulinemia or hypogammaglobulinemia, whereas the number of B and
T lymphocytes is generally normal (1). Death occurs often before adult age secondary to severe recurrent infections. Facial anomalies are a heterogeneous trait in ICF syndrome and mainly include hypertelorism, low-set ears, flat nasal bridge, epicanthal folds and macroglossia (2, 3). Centromeric instability is the most typical feature of the disease. The juxtacentromeric heterochromatin of chromosomes 1, 9 and 16 is markedly undercondensed and is involved in chromosome rearrangements and multiradiate associations, particularly for chromosomes 1 and 16. The instability correlates with a severe hypomethylation of the classical satellites 2 and 3 that are the major components of constitutive heterochromatin (4).

In nearly 60% of ICF cases, mutations in the highly conserved catalytic domain of the DNA methyltransferase 3B gene (DNMT3B, MIM 602900) located at 20q11.2 were identified (5, 6). This observation led to postulate that there are two types of ICF patients who harbor different genetic and epigenetic characteristics. ICF type 1 (ICF1) is characterized by DNMT3B mutations and normal methylation of the alpha satellites. Patients with ICF type 2 (ICF2) are clinically identical to ICF1 patients, but have no DNMT3B mutations and show hypomethylation of the alpha satellites repeats (5). Recently, mutations in ZBTB24 gene were reported in several ICF2 patients (7).

We describe here a Lebanese family where three brothers, affected with ICF2, present a novel deletion in ZBTB24. The pathogenic mechanisms involved in the onset of the disease are discussed.

Materials and methods

Patients and clinical investigations

Three siblings from a Lebanese family were included in our study (Fig. 1). The parents originate from the same village, but deny any relationship between their two families and are not aware of the occurrence of additional affected members outside their nuclear family.

All three patients underwent thorough clinical evaluation, cytogenetic tests, radiological investigations (total body X-rays and brain magnetic resonance imaging), routine blood tests (complete blood count, serum electrolytes, blood glucose levels, triglycerides, cholesterol, thyroid, liver and renal function tests), as well as immunological studies.

Flow cytometry analyses on peripheral blood mononuclear cells (PBMCs) were performed on FACSCaliber (Becton Dickinson, San Jose, CA) and processed using CELLQuest software (Becton Dickinson). PBMCs were stained using either fluorescein isothiocyanate or phycoerythrin conjugated mAbs to CD3, CD4, CD5, CD8, CD16, CD19, CD20, CD25, CD27, CD45RA, CD45RO, CD56, CD57, HLA DR, T-cell receptor (TCR) alpha–beta, TCR gamma–delta. Immunoglobulin classes and immunoglobulin G (IgG) subclasses levels were quantified according to standard techniques.

DNA and cytogenetic studies

After informed consent was obtained from the parents as legal representative of the three affected individuals, ethylenediaminetetraacetic acid and heparin blood samples from all members of the family were collected for genetic studies. DNA was extracted from leucocytes by standard salt-precipitation methods (8).

Cytogenetic studies were performed on cultured peripheral lymphocytes (72-h cultures). Metaphase cells were obtained after PHA short-term cultures. Chromosome slides were made according to routine procedures. Metaphases were analyzed after R-banding.

Mutation analysis

The coding sequences of the following genes were sequenced in one patient, after his DNA was amplified by polymerase chain reaction (PCR): DNMT3B (GenBank Accession Number: NM_006892.3) and ZBTB24 (NM_014797.2). Primers were designed using PRIMER 3 (University of Massachusetts Medical School, Worcester, MA) (http://frodo.wi.mit.edu) and Oligos version 9.3, (University of Helsinki, Fabianinkatu 24, 00100 Helsinki, Finland) and checked for specificity using BLAST (NCBI, 8600 Rockville Pike, Bethesda, MD) (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi). DNA sequences were obtained from UCSC or GenBank databases. PCR reactions were performed using Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA). The amplification conditions for each PCR were 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, the specific annealing temperature for 30 s, and 72°C for 30 s, with a final extension of 10 min at 72°C.

PCR products from genomic DNA were purified using the illustra™ GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK), and both strands of the resultant products were sequenced using the BIGDYE® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) under standard conditions. The labeled products were subjected to electrophoresis on an ABI 3130 Genetic Analyzer sequencing system (Applied Biosystems). Electropherograms were analyzed using SEQUENCE ANALYSIS SOFTWARE version 5.2 (Applied Biosystems).
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Immunological studies

Serum IgM and IgG levels were diminished in all three patients, with serum IgG levels being lowest in the youngest patient (Table 1). Serum IgA levels were in the normal range. Among the IgG subclasses, the IgG3 levels were always elevated for the three brothers.

Absolute and relative numbers of T, B and natural killer cells were within the normal range for age (data not shown). CD19+CD27+ memory B cells were absent in all three children, but present in the father and the mother (Table 1). All the CD27+ cells were equally present in percentages and absolute numbers among the parents and their children. There was a decrease in CD45RO+ memory T cells and an increase in CD45RA+ naïve T cells in both the CD4+ T cells and the CD8+T cell compartments, resulting in increased ratios of naïve: memory cells (Table 1).

Molecular findings

Sequencing of the coding region of DNMT3B gene by fluorescent sequencing in the oldest affected brother (Evani Viegas-Pequignot, unpublished data, 2004) showed no mutation classifying this family as affected with ICF2.

Sanger sequencing of ZBTB24 gene, for the same patient, led to the identification of a 2-bp TA homozygous deletion in the first coding exon of the ZBTB24 gene: c.396_397delTA (Fig. 2). This deletion results in a frameshift and a premature stop codon 19 amino acids downstream (p.His132Glnfs*19; NP_055612.2) and segregates with the phenotype in the studied family. The parents are both heterozygous for the deletion and the three affected brothers are all homozygous for the deletion; suggesting strong linkage to the phenotype.

Table 1. Results of the immunological studies

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Father</th>
<th>Mother</th>
<th>Normal controls</th>
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<tr>
<td>Date of testing</td>
<td>1999</td>
<td>2004</td>
<td>2010</td>
<td>2004</td>
<td>2010</td>
<td>2004</td>
</tr>
<tr>
<td>Serum IgG (mg/dl)</td>
<td>324</td>
<td>716</td>
<td>769</td>
<td>456</td>
<td>533</td>
<td>196</td>
</tr>
<tr>
<td>Serum IgM (mg/dl)</td>
<td>25</td>
<td>&lt;16.8</td>
<td>18.5</td>
<td>41</td>
<td>22.1</td>
<td>30.3</td>
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<tr>
<td>Serum IgA (mg/dl)</td>
<td>&lt;33</td>
<td>195</td>
<td>188</td>
<td>200</td>
<td>176</td>
<td>156</td>
</tr>
<tr>
<td>Serum IgE (IU/ml)</td>
<td>ND</td>
<td>&lt;18.3</td>
<td>ND</td>
<td>&lt;18.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD19+</td>
<td>ND</td>
<td>100</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CD27+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD4+</td>
<td>ND</td>
<td>21</td>
<td>21</td>
<td>37</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>CD45RA+</td>
<td>ND</td>
<td>16</td>
<td>11</td>
<td>15</td>
<td>7</td>
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<tr>
<td>CD45RO+</td>
<td>ND</td>
<td>21</td>
<td>20</td>
<td>28</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>CD8+</td>
<td>ND</td>
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<td>9</td>
<td>10</td>
<td>4</td>
<td>5</td>
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</table>

Ig, immunoglobulin; ND, not determined.
Discussion

The ICF syndrome is a rare genetic autosomal recessive disease. It was first described in the late 1970s. To date, fewer than 50 patients, divided into ICF1 or ICF2, have been reported world-wide (2). Among these 50 patients, 5 cases from three unrelated consanguineous Lebanese families, were found and well characterized at the cytogenetic and molecular levels. In the first family a female patient, born from a second cousin marriage, and was found homozygous for the p.A585V (c.1754C>T) mutation in exon 16 in $\text{DNMT3B}$ gene (10). In the second consanguineous family, a boy aged 13, had a very mild phenotype. In fact, he presented a slight intellectual disability and obesity. No bacterial or viral infections were reported. The methylation studies classified him as an ICF1 patient. This was confirmed at the molecular level: the patient was homozygous for the mutation, p.K770E (c.2308A>G), in exon 21 (Evani Viegas-Pequignot, unpublished data, 2006).

The third family is the subject of this report, where all three siblings were affected with ICF2 and were found to have a novel deletion in the $\text{ZBTB24}$ gene, which has been described lately to be responsible for ICF2 (7).

$\text{DNMT3B}$ mutations are either nonsense mutations, located at the N-terminus of the protein, or missense and splice site mutations that lie mainly in the catalytic domain. The nonsense mutations in $\text{DNMT3B}$ reported to date give rise to a truncated inactive protein and have occurred as compound heterozygous mutations (9). In contrast all but one of the $\text{ZBTB24}$ mutations reported to date, including the deletion identified herein, cause premature stop codons (7). This observation strongly suggests that ICF2 is caused by loss-of-function mutations in $\text{ZBTB24}$.

$\text{ZBTB24}$ belongs to a large family of transcriptional factors. It contains a BTB (bric-a-bric, tramtrack, broad complex)-domain, a DNA-binding A-T hook domain, and eight C2H2 zinc finger domains. Recent studies have shown that $\text{ZBTB24}$ is ubiquitously expressed, with the highest expression levels in naive B cells. Its expression seems to be co-regulated with $\text{DNMT3B}$ during B-cell differentiation, consistent with the decreased immunoglobulin production in ICF patients (7). Importantly, $\text{ZBTB24}$ may play a role in the $\text{BMP2}$ signaling pathway. Indeed, it was shown that $\text{ZBTB24}$ is upregulated by $\text{BMP2}$-induced transcription in murine pluripotent cell lines (11). $\text{BMP2}$ belongs to the transforming growth factor-beta superfamily, which induces bone and cartilage formation (12). $\text{ZBTB24}$ through the regulation of the expression of the $\text{BMP2}$ gene may be responsible for the developmental delay observed in ICF type 2 patients.

$\text{DNMT3B}$ is involved in DNA methylation while till now $\text{ZBTB24}$ function is not well characterized. ICF1 patients have normal methylation pattern of the alpha satellites but hypomethylation of the Xi-CpG islands. ICF2 patients are characterized by a hypomethylation of all the alpha satellites but normal methylation of
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References