CASE REPORT
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Immunodeficiency, Centromeric Region Instability, and Facial Anomalies Syndrome (ICF) in a Boy with Variable Clinical and Immunological Presentations

Mohammad Hassan Bemanian[1], Saba Arshi[1], Mohammad Nabavi[1], Mohammad Vafaee-Shahi[2], Morteza Fallahpour[1], Sima Shokri[1], Afshin Rezaeifar[1], Hossein Shahzadi[3], and Fatemeh Atashrazm[1]

[1] Department of Allergy and Clinical Immunology, Iran University of Medical Sciences, Tehran, Iran
[2] Pediatric Growth and Development Research Center, Iran University of Medical Sciences, Tehran, Iran
[3] Department of Pediatric Cardiology, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome is a rare primary immunodeficiency disorder characterized by recurrent infections and low immunoglobulin levels due to variable combined immunodeficiency, and centromeric region instability, and facial dysmorphism.

We describe a 12-year-old boy with recurrent respiratory tract infections, facial anomalies, scoliosis, and psychomotor retardation. He had recurrent pneumonia with low serum IgG and IgM levels during infancy and preschool age. Later at the age of 10, he developed recurrent ear infections. An IgA and IgM deficiency was found accompanied by a normal B-cell and T-cell count as well as an impaired candida-induced T-cell proliferation. Further evaluations revealed a missense mutation in the *DNMT3B* gene on chromosome 20. Chromosomal analysis showed a sunburst multi-radial feature on chromosome 1, which is a hallmark of ICF syndrome.

The genetic mutation and chromosomal abnormality along with clinical findings are compatible with the diagnosis of ICF syndrome. To the best of our knowledge, this is the first time that scoliosis is observed in an ICF patient.

The additional variable clinical symptoms in the case were the presence of spastic gait as well as hypogammaglobulinemia with immunoglobulin isotype switch at different ages.

Keywords: Chromosomal instability; DNA methyltransferase 3B; Immunodeficiency; Scoliosis

INTRODUCTION

Immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome is a rare genetic immunodeficiency disorder, inherited in an autosomal recessive manner. Patients with ICF present immunodeficiency with low levels of immunoglobulins, facial dysmorphism, and high Frequency of chromosomal abnormalities, and instability of peri-centromeric regions of certain

Corresponding Author: Fatemeh Atashrazm, MD; Department of Allergy and Clinical Immunology, Iran University of Medical Sciences, Tehran, P.O.Box: 1445613131, Iran.
Tel: (+98 21) 6655 4933, Fax: (+98 21) 6651 7118, E-mail: dr.atashrazm@gmail.com

chromosomes.[1]

The chromosomal instability results from DNA hypomethylation in the heterochromatin site next to the centromeres of chromosomes 1, 9, and 16. Karyotype analysis often shows multiradial, deletion, and duplication of the above-mentioned chromosomes and sometimes chromosome 7.[2] The leading cause of ICF syndrome is mutations in genes whose products play role in DNA methylation. The majority of the patients have mutations in *DNMT3B* (DNA methyl-transferase 3B) gene on chromosome 20. This type of disease is called ICF1. In other cases, described as ICF2, the mutation occurs in the *ZBTB24* gene located on chromosome 6. ZBTB24 is a transcription factor, which coordinates with DNMT3B to regulate DNA methylation. ICF2 patients seem to have a similar phenotype as ICF1 patients. A small group of cases with the unknown genetic mutation is also described as ICFX.[3]

Patients with ICF are subjected to recurrent infections and immunodeficiency especially humoral immune system defects, which manifest as a reduction in immunoglobulins. Although, B-lymphocyte count is normal.[4] Most of the patients have facial malformations including hypertelorism, epicanthal fold, flat nasal bridge, and low set ears. Many ICF cases have psychomotor retardation and the cases have variable intelligence with some with moderate to severe mental retardation.[5]

In this paper, we report the case of a now 12-year-old patient with recurrent infections and developmental delay.

CASE REPORT

We describe a 12- year-old boy of consanguineous Iranian parents, with a long history of immune deficiency symptoms since infancy. He was born full-term and developed kidney stones at the age of 7 months. At 9 months of age, the boy was presented with a high fever and cough and was admitted to the hospital because of pneumonia. Until the age of 14 months, he was hospitalized four times due to lower respiratory tract infections. There was a family history of repeated abortions and early deaths due to unknown causes. He has three brothers; one of whom underwent thyroid lobectomy due to thyroid nodule at 20 years of age and another one had arthritis in the rheumatoid state.

At 14 months of age, he was evaluated for recurrent infections. His immunologic investigations showed hypogammaglobulinemia (decreased IgG and IgA levels) (Table 1) and Specific antibody responses were abnormal at the age of 2 years. The IgG subclasses were in normal ranges. Flow cytometry analysis revealed normal B-cell and T-cell count. Sweat chloride testing for cystic fibrosis was performed and the result was normal. He received intravenous immunoglobulin, which was continued until the age of 3 years. In this period, he was once admitted to the hospital for pneumonia and once with the possibility of osteomyelitis.

At the age of 3 years old, he was re-evaluated and investigations showed normal immunoglobulin levels. Hence, immunoglobulin replacement therapy was discontinued. At 5 years of age, he was admitted to the hospital two times; once due to pneumonia and once for gingivostomatitis. Immunoglobulins were re-checked and they were in normal ranges.

He also had psychomotor developmental delay. He was able to sit at 18 months and started walking when he was 3 years old. By the age of 2, he was able to speak. He had facial anomalies including hypertelorism, epicanthic folds, flat nasal bridge, and mild left ptosis.

The patient also showed signs of skeletal deformity in the form of lumbar scoliosis (Figure 1) and had to limp with a spastic gait. Because of abnormal gait, ptosis, and lower limb spasticity, further investigations were performed.

A brain MRI was conducted, which was normal. Muscle enzymes were also in normal ranges.

Table 1.Serum gamma-globulin levels of the patient at different age points

Immunoglobuin	mg/dL	Normal range (mg/dL)
Levels at 14 months		
IgA	38	26-74
IgG	**347**	553-971
IgM	**26**	35-81
Levels at 10 years		
IgA	**25**	71-191
IgG	1225	889-1359
IgM	**19**	46-112

Electromyography (EMG) and nerve conduction velocity (NCV) were normal. But his bone scan showed increased uptake in his left hip and both knees that were suspected to be autoimmune related. Rheumatology and neurology assessments were conducted and the results were normal. His liver enzymes at 11 years of age were mildly elevated and abdominal sonography revealed grade 1 fatty liver disease. Until his 10th year of age, he had no significant infection; however, he later developed recurrent bilateral draining ear infections. His immunoglobulin levels were re-checked and indicated IgA and IgM deficiency (Table 1). Flow cytometry analysis showed normal lymphocyte count. A lymphocyte transformation test was performed and lymphocyte response to phytohemagglutinin and BCG was normal but he had impaired response to candida.

For genetic testing, we performed whole-exome sequencing to identify the most relevant mutations that might explain the disease in this patient. Whole exome sequencing was performed using Illumina NovaSeq6000 using Agilent SSV7 library kit. Interestingly, a homozygous mutation was identified in the *DNMT3B* gene on chromosome 20q11.21, which was a T>A substitution at position c.2166 on exon 20. This substitution caused a missense mutation leading to an amino acid change of p.D722E. This mutation was following patient clinical presentations and thus a diagnosis of ICF1 was made.

The genetic findings were then confirmed by karyotype analysis on peripheral blood. The cytogenetic analysis showed an abnormal male chromosome complement with a “windmill” or “starburst” multi-radial chromosome 1 (Figure 2). These findings were consistent with the clinical diagnosis of ICF syndrome with an apparent rosette structure that mainly involved the centromere of chromosome 1.

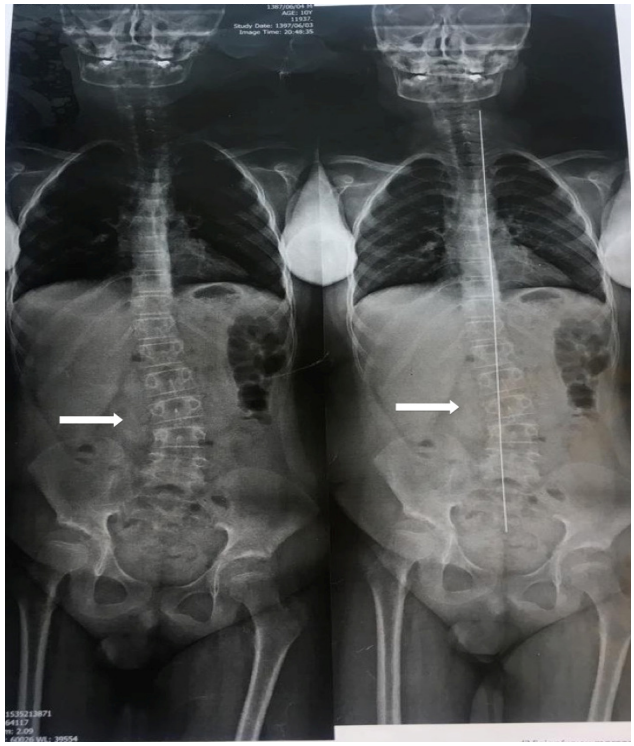

Figure1.Thoracolumbar Spine X-Ray of the patient demonstrating scoliosis

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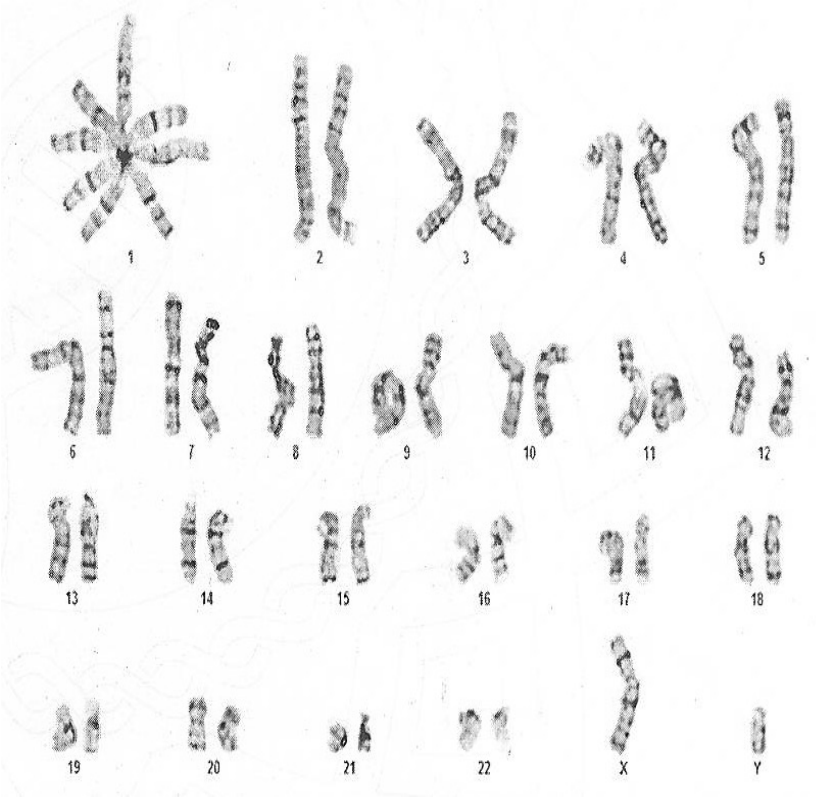

Figure 2. Conventional cytogenetic study on peripheral blood lymphocytes: A starburst multi-radial feature is seen in chromosome 1

DISCUSSION

Immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome is a rare autosomal recessive disorder most often arising from a homozygous mutation in DNA methyltransferase 3B gene*DNMT3B*. Methylation is important for gene expression and is essential for embryonic development and chromosomal stability.[6] Hence mutations in *DNMT3B*, which result in its reduced enzymatic activity, lead to DNA hypomethylation across the genome. In a study by Jin et al*,* global gene expression analysis in cases of ICF revealed changes in the expression of 778 genes, many of which were critical for immune function, development, and neurogenesis.[7] These findings are consistent with clinical manifestations of the disease including combined immunodeficiency, chromosomal pericentromeric anomalies, and mild facial dysmorphism, and psychomotor retardation.[8]

Here, we present a boy with ICF-1, with a mutation in the *DNMT3B* gene. *DNMT3B* is located on the long arm of chromosome 20 at position 20q11.2. It contains 23 exons and 22 introns, which encodes an enzyme of 853 amino acid residues with methyltransferase activity.[9] The N terminal region of DNMT3B protein is the regulatory unit and is mainly involved in enzyme localization and protein-protein interaction. The C terminal area however contains the main catalytic region with 6 catalytic motifs that are involved in the target recognition site, binding to the substrate cytosine, and transferring the methyl group to the target site in DNA. Exons 16 to 23 encode the C-terminal region. Not surprisingly, the majority of missense mutations in *DNMT3B* found in ICF are located in these exons leading to changes in amino acid residues located in the catalytic region. These include the well-known mutations Ala585Thr[10] and Ala603Thr[11] on exons 16 and 17. However, mutations in other parts of the *DNMT3B* gene affecting the N terminal region have also been observed in ICF cases.[12] Similar to the majority of the reported cases, our patient had a

missense mutation in the catalytic C terminal region of the protein, which caused p.D722E substitution. This mutation has been previously reported in another study in an Iranian population.[13] In this study, Yazdani et al, studied 550 patients with predominantly antibody deficiency. Twelve out of these 550 cases were shown to be ICF cases, 7 of whom were ICF1 with *DNMT3B* mutation. Interestingly, 6 out of 7 of these cases had homozygous p.D722E mutations similar to our patient.

One of the main features of ICF is the presence of cytogenetic abnormalities. Since *DNMT3B* mutations caused decreased enzymatic activity, the heavily mutated regions of DNA are strikingly affected in ICF patients. The heterochromatin region of chromosomes 1, 9, and 16 contain a high frequency of these hypermethylated regions compared to other parts of the genome.[14] Also the heterochromatin is generally long in chromosomes 1, 9, and 16, which make them more prone to breakage. Thus hypo-methylation of heterochromatin leads to breaking and re-joining of the chromosomes and generating multi-radial configurations with the presence of multiple p and q arms. In the presented case, the patient showed a starburst multi-radial feature in chromosome 1, consistent with previous reports. But chromosomes 9 and 16 showed normal characteristics. In a study conducted by Kamae et al in Japan, 9 out of 11 ICF patients showed chromosomal abnormalities at 1qh and/or 16qh.[15] These chromosomal aberrations are pathognomonic for ICF1 syndrome and confirm the diagnosis.[16]

Developmental and neurological problems are two of the main clinical manifestations reported in ICF syndrome. Almost half of the patients have been shown to have a motor delay and most of them have a speech delay.[17] In addition, the intelligence quotient is normal in more than one-third of the patients.[8] The case presented here also had a delay in talking and motor skills. But he had no intellectual disability.

Like most other ICF patients, our patient also had facial dysmorphism. Interestingly, he had scoliosis with a pelvic tilt that produces abnormal gait for the patient, although following detailed investigations no justification could be made for his skeletal deformity. In previous studies, hip dislocation has been reported in one patient[17] but to the best of our knowledge, this is the first time that scoliosis is observed in an ICF patient.

Recurrent infection is a common presentation of ICF syndrome, in which respiratory and gastrointestinal systems are mainly involved. Almost all of the patients have been reported to suffer from recurrent respiratory infections.[5] Recurrent infections most often occur in the first year of life and usually are the first manifestation of the disease. Our patient had recurrent pneumonia in early childhood and recurrent otitis media in adolescence. He also suffered from herpetic stomatitis when he was 5 years old. However, He had no gastrointestinal complications.

Infections in ICF patients are due to hypogammaglobulinemia or combined immunodeficiency. Patients have been shown to have variable immunoglobulin deficiency as reductions in serum IgG, IgG subclasses, IgA, and/or IgM levels can be seen. Almost all patients with ICF do not have memory B-cells.[18,19] Interestingly, the hypogammaglobulinemia in our patient showed a switch in the class of immunoglobulin across different time points as he had reduced IgG and IgM levels in early childhood, which later became normal at pre-school age, and further presented low levels of IgM and IgA but normal levels of IgG at 10 years of age.

In ICF patients T-cell level is mostly normal in early childhood but it may decrease in many cases until adulthood.[20] T-cell defects have been seen in several patients, which can justify opportunistic infections such as pneumocystis jiroveci and candida in some patients. Our patient had a normal T-cell count, but the T-cell response to candida was impaired although there was no clinical manifestation such as candida infection.

We reported a case of ICF1 with a missense mutation in the *DNMT3B* gene, which resulted in an amino acid change in the catalytic domain of the DNMT3B protein. Interesting clinical symptoms in the case were the presence of scoliosis, pelvic tilt, and spastic gait as well as hypogammaglobulinemia with immunoglobulin isotype switch at different ages.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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