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LETTER TO EDITOR

The Value of Chromosome Analysis to Interrogate Variants in *DNMT3B* Causing Immunodeficiency, Centromeric Instability, and Facial Anomaly Syndrome Type I (ICF1)

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To the Editor:

Immunodeficiency, centromeric instability, facial anomalies syndrome (ICF) is a rare autosomal recessive disorder and is one of the few heritable human diseases caused by mutations in a DNA methyltransferase [1]. The majority of patients with ICF syndrome have a defect in either the *DNMT3B* or *ZBTB24* gene, classified as ICF1 and ICF2, respectively [2]. DNMT3B, in conjunction with DNMT3A and the catalytically inactive stimulatory factor DNMT3L, is responsible for de novo DNA methylation during embryogenesis and early development [3]. DNMT3A and DNMT3B are responsible for methylating unique genomic targets, with DNMT3B being solely responsible for methylating cytosine residues of CpG dinucleotides in highly repetitive satellite 2 and 3 DNA of pericentromeric heterochromatin [3]. Patients with biallelic pathogenic variants in *DNMT3B* show hypomethylation and characteristic aberrations of the pericentromeric regions of chromosomes 1, 9, and 16 in mitogen-stimulated lymphocytes. The most common clinical observations in ICF1 syndrome are hypogammaglobulinemia, intrinsic T cell defects leading to increased risk of opportunistic infections, neurologic abnormalities, and characteristic facial features including epicanthal folds, hypertelorism, flattened nasal bridge, low set ears, micrognathia, and macroglossia. Many patients with ICF1 die by early adulthood due to infectious diseases [2].

Here we describe a 14-month-old Caucasian female born full term to nonconsanguineous parents with no facial dysmorphism. She was well until 3 months of age when she began having increased work of breathing and failure to thrive with diarrhea. At 4 months, she developed rhinovirus and enterovirus infection necessitating hospitalization in the intensive care unit. At 5 months of age, she developed enteroviral meningitis, prompting an immune evaluation that demonstrated agammaglobulinemia with normal numbers of total T and B cells (Supplemental Table I). She was successfully treated with high-dose immune globulin and the investigational antiviral drug pocapavir, after which she was continued on immune globulin replacement. However, she continued to have unexplained diarrhea, and at 6 months of age developed *Pneumocystis jiroveci* pneumonia. She was successfully treated and maintained thereafter on trimethoprim-sulfamethoxazole prophylaxis. Although she did not develop any additional serious infections, she continued to have diarrhea, poor feeding, and poor weight gain, resulting in gastrostomy tube placement at 7 months. In addition, she experienced a developmental delay in her milestones most notably unable to walk any steps by 14 months or say any words.

Genetic testing revealed two heterozygous missense variants which were reported as variants of uncertain clinical significance in the *DNMT3B* gene (NM_006892.3), c.2177T>G and c.2281G>C, in this patient. Parental studies confirmed they are in the compound heterozygous state (i.e., *in trans*). The first missense variant (c.2177T>G) occurs in exon 20 and results in a substitution of glycine for valine at codon 726

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(p.Val726Gly). This variant is approximate to the C-terminal of C-5 methyltransferase domain and has been reported in the homozygous and compound heterozygous state in three patients from two unrelated families affected with ICF syndrome [4, 5]. This variant is reported in the genome aggregation database (gnomAD) with a minor allele frequency (MAF) in the non-Finnish European control population of 0.0031% (4/129,200 alleles), with no homozygotes reported. The second missense change (c.2281G>C) is located in exon 21, which results in a substitution of glutamic acid with glutamine at codon 761 (p.Glu761Gln). This variant occurs at a highly conserved amino acid residue and is close to the C-terminal of the C-5 methyltransferase domain. In the gnomAD database, the MAF of this variant in the non-Finnish European control population is 0.0031% (4/129,158 alleles), with no homozygotes reported. This variant is rare and only observed in 4 out of 282,846 alleles. Thus far, this variant has not been reported in the literature in association with *DNMT3B*-related diseases. Computational predictions suggest potentially damaging effects of this change.

In addition, genetic testing also identified a heterozygous missense variant in the *TNFRSF13B* gene (NM_012452.2), c.542C>A (p.Ala181Glu), encoding transmembrane activator and CAML interactor (TACI), that was classified as likely pathogenic in this patient. This particular change has been observed both in patients affected with common variable immunodeficiency (OMIM No. 604907) and also in normal individuals. Parental testing indicated this variant was inherited from the patient's mother who appears to be normal, with a normal IgG (1203 mg/dl) and no history of primary immunodeficiency or recurrent infections.

Inspired by the findings of the biallelic mutations in *DNMT3B*, chromosomal analysis was performed from the patient's blood sample to determine the pathogenic nature of these *DNMT3B* variants. Lymphocytes were cultured in phytohemagglutinin-stimulated RPMI media for 72 h and cell cultures were synchronized 90 min prior to harvesting with colcemid. Harvesting, slide preparation, and Giemsa banding through trypsin digestion were carried out according to standard clinical laboratory protocols. Characteristic chromosomal aberrations in ICF1 syndrome were observed in 16 out of 20 cells analyzed from two separate cultures. Pericentromeric aberrations of chromosomes 1, 9, and 16 included centromeric decondensation, whole-arm deletions, translocations, isochromosomes, and multi-radial configurations (Fig. 1). These distinct aberrations specific to chromosomes 1, 9, and 16 are pathognomonic for ICF1 syndrome, thus verifying the diagnosis and pathogenic nature of both *DNMT3B* mutations.

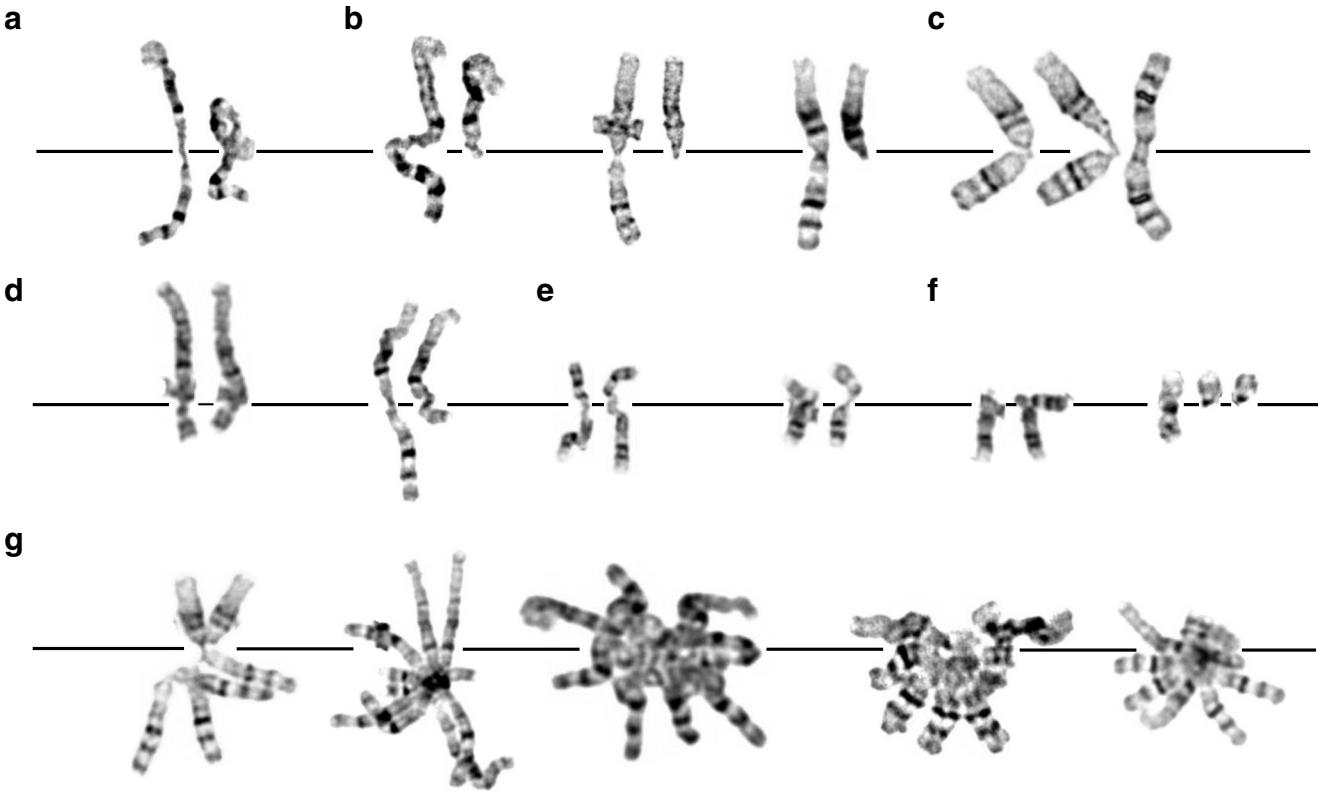

Fig. 1 Chromosomal aberrations. **a** Decondensation of the pericentromeric region of chromosome 1. **b** Whole-arm deletion of the long arm of chromosome 1. **c** Isochromosome consisting of two copies of the long arm of chromosome 1 (third copy of chromosome 1). **d** Translocation between the short arm of chromosome 1 and the short arm of chromosome 16. **e** Decondensation of the pericentromeric region of chromosomes 9 and 16, respectively. **f** Whole-arm deletions of the short arm of chromosome 9 and the long arm of chromosome 16, respectively. **g** Multi-branched radials

This case highlights the importance of interrogating novel *DNMT3B* variants through routine chromosome analysis, which is widely available in clinical cytogenetics laboratories. While cytogenetic analysis along with familial testing enabled reclassification by the commercial testing laboratory of only the c.2177T>G (p. Val726Gly) variant from VUCS to pathogenic, both approaches were essential in establishing the diagnosis of ICF1 syndrome in this patient. Making this diagnosis was significant and changed clinical management. Poor outcomes can be observed in patients with ICF syndrome related to complications of immune deficiency. Allogeneic hematopoietic stem cell transplantation is a treatment option and was only considered for this patient after the diagnosis was clear [2]. This patient recently underwent HCT, and fortunately is doing well and receiving routine post-transplant care as an outpatient.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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