Universiteit Leiden
The Netherlands

Expanding the mutation spectrum in FSHD and ICF syndrome

Boogaard, T.L. van den

Citation
Boogaard, T. L. van den. (2018, February 13). *Expanding the mutation spectrum in FSHD and ICF syndrome*. Retrieved from https://hdl.handle.net/1887/60938

Version:	Not Applicable (or Unknown)
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/60938

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/60938 holds various files of this Leiden University dissertation.

Author: Boogaard, T.L. van den
Title: Expanding the mutation spectrum in FSHD and ICF syndrome
Issue Date: 2018-02-13

Marlinde L van den Boogaard[1,+], Peter E Thijssen[1,2,+], Caner Aytekin[3], Francesco Licciardi[4], Ayça A Kiykim[5], Lucia Spossito[6], Virgil ASH Dalm[7,8], Gertjan J Driessen[9,10], Rogier Kersseboom[11,12], Femke de Vries[11], Monique M van Ostaijen-ten Dam[13], Aydan Ikinciogullari[14], Figen Dogu[14], Matias Oleastro[6], Edgardo Bailardo[15], Lucia Daxinger[1], Ercan Nain[5], Safa Baris[5], Maarten JD van Tol[13], Corry Weemaes[16,] Silvère van der Maarel[1]

1 Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
2 Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands
3 Department of Pediatric Immunology, Dr Sami Ulus Maternity and Children's Research and Educational Hospital, Ankara, Turkey
4 Department of Paediatrics II, Regina Margherita Hospital Città della Salute e della Scienza di Torino, Torino, Italy
5 Pediatric Allergy and Immunology, Marmara University Pendik Training and Research Hospital, Istanbul, Turkey
6 Department of Immunology and Rheumatology, Hospital "J.P Garrahan", Buenos Aires, Argentina
7 Department of Immunology, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands
8 Department of Internal Medicine, Division of Clinical Immunology, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands
9 Department of Paediatric Infectious Diseases, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands
10 Department of Pediatrics, Juliana Children's Hospital/Haga Teaching Hospital, The Hague, the Netherlands
11 Department of Clinical Genetics, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands
12 Medical service, Stichting Zuidwester, Middelharnis, The Netherlands
13 Department of Pediatrics, Laboratory Immunology, Leiden University Medical Center, Leiden, The Netherlands
14 Department of Pediatric Immunology and Allergy, Ankara University School of Medicine, Ankara, Turkey
15 Department of Genetics, Hospital "J.P. Garrahan", Buenos Aires, Argentina
16 Department of Pediatric Infectious Diseases and Immunology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
+ These authors contributed equally

6

EXPANDING THE MUTATION SPECTRUM IN ICF SYNDROME: EVIDENCE FOR A GENDER BIAS IN ICF2

Van den Boogaard et al. 2017, Clinical Genetics Oct;92(4):380-387

ABSTRACT

Background: Immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome is a rare, genetically heterogeneous, autosomal recessive disorder. Patients suffer from recurrent infections caused by reduced levels or absence of serum immunoglobulins. Genetically, 4 subtypes of ICF syndrome have been identified to date: ICF1 (*DNMT3B* mutations), ICF2 (*ZBTB24* mutations), ICF3 (*CDCA7* mutations), and ICF4 (*HELLS* mutations).

Aim: To study the mutation spectrum in ICF syndrome.

Materials and methods: Genetic studies were performed in peripheral blood lymphocyte DNA from suspected ICF patients and family members.

Results: We describe 7 ICF1 patients and 6 novel missense mutations in *DNMT3B*, affecting highly conserved residues in the catalytic domain. We also describe 5 new ICF2 patients, one of them carrying a homozygous deletion of the complete *ZBTB24* locus. In a meta-analysis of all published ICF cases, we observed a gender bias in ICF2 with 79% male patients.

Discussion: The biallelic deletion of *ZBTB24* provides strong support for the hypothesis that most ICF2 patients suffer from a *ZBTB24* loss of function mechanism and confirms that complete absence of *ZBTB24* is compatible with human life. This is in contrast to the observed early embryonic lethality in mice lacking functional Zbtb24. The observed gender bias seems to be restricted to ICF2 as it is not observed in the ICF1 cohort.

Conclusion: Our study expands the mutation spectrum in ICF syndrome and supports that *DNMT3B* and *ZBTB24* are the most common disease genes.

INTRODUCTION

Immunodeficiency, centromeric instability, and facial anomalies (ICF; OMIM #242860/#614069) syndrome is a rare, autosomal recessive disorder. Patients suffering from this characteristic triad have a severely decreased life expectancy if left untreated. In ICF syndrome, the primary immunodeficiency is characterized by an absence or strong reduction of serum immunoglobulins in the presence of circulating B-cells, leading to recurrent and eventually fatal infections of mainly the gastro-intestinal and respiratory tracts[1; 2]. Although humoral immunodeficiency is pronounced in patients with ICF syndrome, opportunistic infections observed in about 20% of the patients also suggest involvement of a T-cell dysfunction[2]. Currently, patients are most often treated by intravenous immunoglobulin therapy and few cases underwent successful hematopoietic stem cell transplantation[1-4]. ICF syndrome patients present with a distinct but variable set of facial anomalies, including, but not limited to, hypertelorism, flat nasal bridge, and epicanthus[1; 2].

The cytogenetic hallmark of ICF syndrome is centromeric instability. Upon phytohemagglutinin stimulation of lymphocytes, aberrant chromosomal configurations can be observed in metaphase spreads. These aberrant configurations mainly involve the centromeric regions of chromosomes 1, 9, and 16 and correlate with CpG hypomethylation of repeats located in these (peri)centromeric regions[5; 6]. The combination of a compromised humoral immune response, the abnormal facial appearance, and cytogenetic abnormalities is diagnostic for all ICF syndrome patients.

ICF syndrome is a genetically heterogeneous disease. In recent years the number of disease genes has expanded from one to four, with still few cases left unexplained. About half of the cases carry biallelic mutations in the DNA methyltransferase 3B (*DNMT3B*; ICF1) gene, the first identified disease gene[7; 8]. ICF1 patients most often carry missense mutations in the catalytic methyltransferase domain of *DNMT3B*, leading to partial loss of function. Nonsense mutations in *DNMT3B* are only found in combination with missense mutations, suggesting that complete loss of *DNMT3B* activity is not compatible with life, which is consistent with *Dnmt3b* knock-out studies in mice[2; 9].

We and others have described mutations in the zinc finger and BTB (Broad-Complex, Tramtrack, and Bric a Brac) domain containing 24 (*ZBTB24*; ICF2) gene in some patients negative for mutations in *DNMT3B*[2; 4; 10-14]. Most often, homozygous nonsense mutations were identified, which are predicted to lead to an absence of full length protein in patients. In addition, a few ICF2 cases with homozygous or compound heterozygous missense mutations in *ZBTB24* have been described. More recently, missense mutations in the cell division cycle associated 7 (*CDCA7*; ICF3) gene and splice site, missense, and

nonsense mutations in the helicase, lymphoid specific (*HELLS*; ICF4) gene were found to underlie ICF syndrome in almost all patients negative for mutations in *DNMT3B* and *ZBTB24*[15].

Here we report the identification of seven ICF1 cases with previously unreported mutations in *DNMT3B*. Additionally, we present five ICF2 cases, carrying both known and novel mutations, including one case with a homozygous deletion of the complete *ZBTB24* gene. With these new cases included, the total number of genetically confirmed ICF cases reported to date is 77 (including patient 2 from Franceschini *et al*.[16] with *ZBTB24* mutations (unpublished F.Licciardi)), of which 56% have mutations in *DNMT3B*, 31% have mutations in *ZBTB24*, and the remaining 13% have mutations in *CDCA7* or *HELLS*. This study, combined with earlier mutation reports[1; 2; 4; 11; 12; 15; 17-21], also revealed a gender bias specific to the ICF2 population with 79% of all ICF2 patients being male. In summary, we expand the mutation spectrum for the two most common genetic causes of ICF, show that complete absence of the *ZBTB24* locus is compatible with human life, and identify a gender bias in ICF2.

MATERIALS AND METHODS

Patients

Nine ICF families were studied after informed consent and the study protocol was approved by the relevant institutional review boards. All patients fit the immunological, cytogenetic, and clinical criteria for ICF syndrome. Detailed clinical and immunological information is provided in the results section and in the Tables S1 and S2.

Sanger sequencing of ICF disease genes

For all index cases, *DNMT3B* and/or *ZBTB24* variant analysis was performed by Sanger sequencing after polymerase chain reaction (PCR) amplification. For *DNMT3B* the exons encoding the catalytic domain (exons 16-23) were analysed using intronic primers at a position of at least 50 nucleotides from the splice donor or acceptor site. For *ZBTB24* all coding exons (exons 2-7) were analysed using intronic primers at a position of at least 50 nucleotides from the splice donor or acceptor site. Primers and amplification conditions were published previously[10; 22]. For exon 19 of *DNMT3B* a new primer pair was used (DNMT3Bex19fwd2: 5'-ACTGGTAGGCATCACCCTGA-3' and DNMT3Bex19rev2: 5'-CACCCACCAATCATCACTGC-3'). The *DNMT3B* genomic sequence was obtained from Ensemble (build 37) [GRCh37:20:31350191:31397162] (Genomic Refseq: NG_007290.1, Transcript Refseq: NM_006892.3). The *ZBTB24* genomic sequence was obtained from Ensemble (build 37) [GRCh37:6:109781719:109806439] (Genomic Refseq: NG_029388.1,

Transcript Refseq: NM_014797.2). The functional consequences of variants were predicted using SIFT[23], Mutation Taster[24], PolyPhen-2[25], and PROVEAN[26]. Identified variants have been submitted to the Leiden Open Variant Database (http://databases.lovd.nl/shared/genes/DNMT3B; http://databases.lovd.nl/shared/genes/ZBTB24).

SNP array analysis

For family Rf1875 a single nucleotide polymorphism (SNP) Array analysis was performed with the Human Cyto 12-SNP genotyping array (Illumina, San Diego, California, USA), according to the manufacturer's instructions, to identify the breakpoints of the chromosome 6 deletion.

Statistical analysis

For the review of patient numbers and gender distributions we only included genetically confirmed cases with sufficient clinical follow up (including patient 2 from Franceschini *et al*.[16] with *ZBTB24* mutations (unpublished F.Licciardi)). A possible gender bias in the different subtypes of ICF was analysed using a non-parametric Chi-square test (SPPS version 23, IBM) under the null hypothesis of equal distribution between genders, which is expected for autosomal recessive disorders. Two confirmed ICF1 patients were added to the analysis as missing values because of discrepancies in gender between different publications they were reported in (PB and PM from Jiang et al.)[1; 21; 27].

RESULTS

Clinical description of ICF syndrome patients

All twelve ICF patients that we describe in this report display facial dysmorphism characteristics and delayed development as previously described (Table S1). Recurrent infections were frequent: six out of seven ICF1 patients and all five ICF2 patients had suffered from bronchopneumonia, and one ICF1 patient and two ICF2 patients had suffered from meningitis. Until now, opportunistic infections, such as Candida and *Pneumocystis jirovecii*, have not been reported in the ICF1 patients in this study and were only observed in one ICF2 case (Table S1). Almost all ICF2 patients reported previously display a variable degree of intellectual disability[2; 12]. Among the ICF2 patients reported in this study there is one with normal intelligence, whereas the others showed intellectual disability (Table S1). At first analysis and before initiation of intravenous immunoglobulin substitution (IVIG), all evaluable ICF1 and ICF2 patients displayed (strongly) decreased serum IgG and IgM levels, except for one ICF1 patient having a normal IgM concentration (Table S2). IgA showed a normal level in one ICF2

case and was (strongly) decreased in the remaining seven evaluable ICF patients. After diagnosis, all patients were treated with IVIG. Peripheral blood B cells were present in normal numbers in six out of seven ICF1 patients and one out of five ICF2 patients and (strongly) decreased in the remaining cases.

ICF1 patient mutations

In seven ICF patients from four different families, mutations in *DNMT3B* were identified (Fig. 1A). In total, six missense mutations in *DNMT3B* were found, which have not previously been reported in ICF syndrome. Five of them have not previously been described in dbSNP, the National Heart, Lung, and Blood Institute Exome Sequencing Project (ESP) Exome Variant Server or the Exome Aggregation Consortium (ExAC) Browser. The mutation *DNMT3B* c.2221G>A (p.G741R) has been described heterozygously in three out of 121,408 exomes in the ExAC database and dbSNP (rs769823434), a frequency concordant with the recessive nature of ICF syndrome.

All six missense mutations affect highly conserved residues in the catalytic domain of DNMT3B (Fig 1B). Each missense mutation is predicted as damaging or deleterious by at least two out of four prediction tools (Table S3). In two ICF1 families the patients (Rf1648-2.1, Rf1771-2.1, 4.2, and 4.3) carry a homozygous missense mutation in *DNMT3B* (Fig. 1C, 1D, S1A and S1B). In the two other ICF1 families the patients (Rf1840-2.1 and 2.2, Rf1693-2.4) carry compound heterozygous missense mutations (Fig 1E, 1F, S1C and S1D) in *DNMT3B*. All homozygous or compound heterozygous mutations were identified in both or only one parent, respectively, as expected for the recessive inheritance pattern of ICF syndrome (Table S1).

ICF2 patient mutations

In four ICF2 patients from four different families we identified nonsense mutations in *ZBTB24*. In a fifth family a microdeletion affecting several genes, including the complete *ZBTB24* locus, was identified (Fig. 2A). The affected individuals from the non-consanguineous family Rf1225 and the consanguineous families Rf1461 and Rf1462 carry a one base pair deletion on both alleles, which is predicted to create a frameshift and a premature stopcodon in ZBTB24 (Fig. 2B, 2C, 2D, S2A, S2B and S2C). Their parents are all heterozygous carriers of this mutation. This mutation has been previously described in one other ICF2 patient[10]. The ICF2 patient Rf1759B-2.1 (case 1 in Franceschini *et al*. 1995[16]) carries a homozygous duplication in *ZBTB24* (Fig. 2E and S2D), which creates a premature stopcodon. Both non-consanguineous parents are heterozygous carriers of this mutation. This mutation is described heterozygously in one out of 121,392 exomes in the ExAC database and dbSNP (rs770082593), again in concordance with the rare and recessive nature of ICF syndrome. Rf1875 is a consanguineous family (Fig. 2F, 2G),

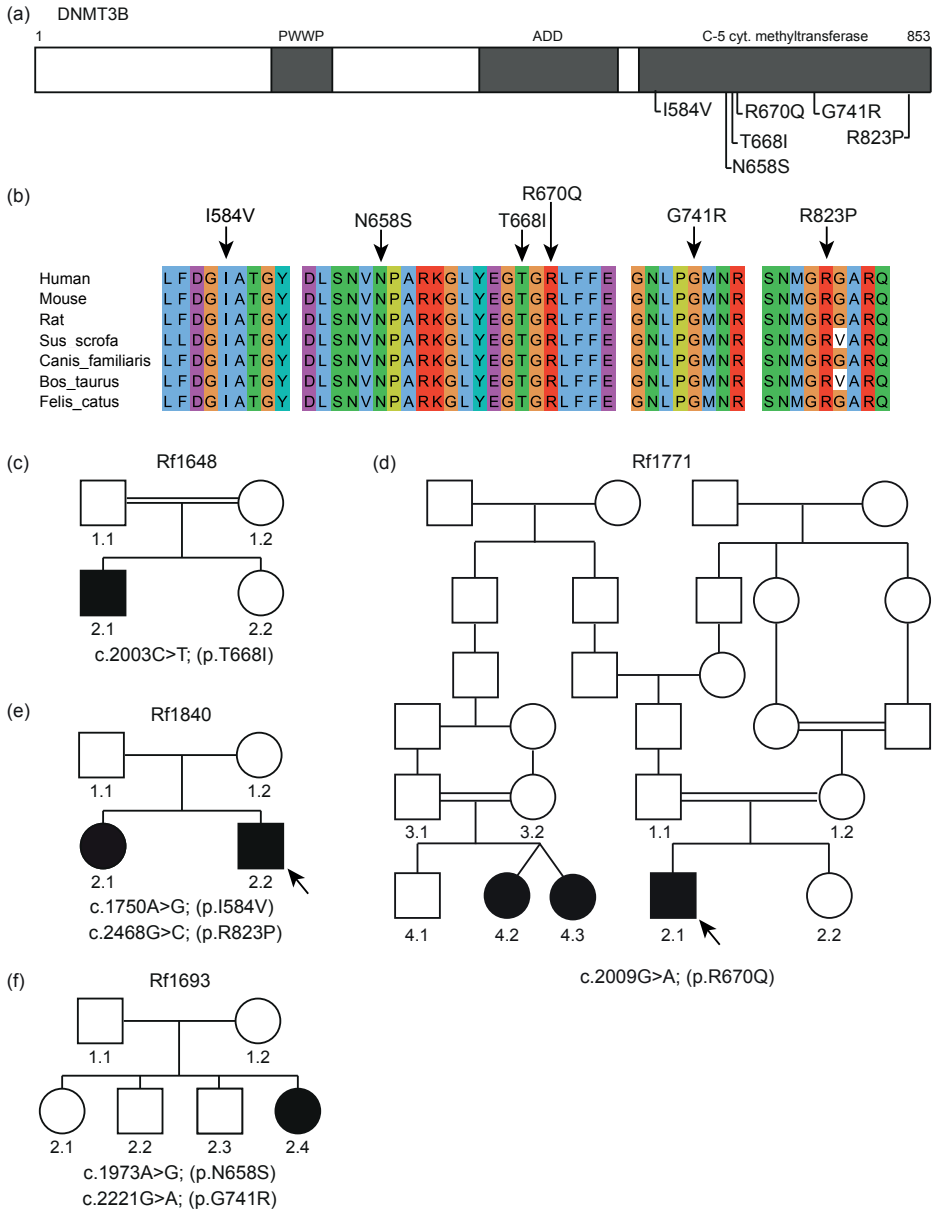


FIGURE 1. DNMT3B mutations in ICF1 families. **(a)** Schematic representation of the DNMT3B protein (GenBank: NP_008823.1). The identified amino acid changes of the ICF1 families are indicated. **(b)** Multiple-sequence alignment (MSA) of DNMT3B across distinct species for DNMT3B mutations in the ICF1 families. MSA was performed with ClustalOmega and alignment was viewed in Jalview and colored as in ClustalX. **(c-f)** Overview of the ICF1 pedigrees and their *DNMT3B* mutations. For families with multiple ICF patients, probands are indicated with an arrow. ICF, immunodeficiency, centromeric instability, and facial anomalies.

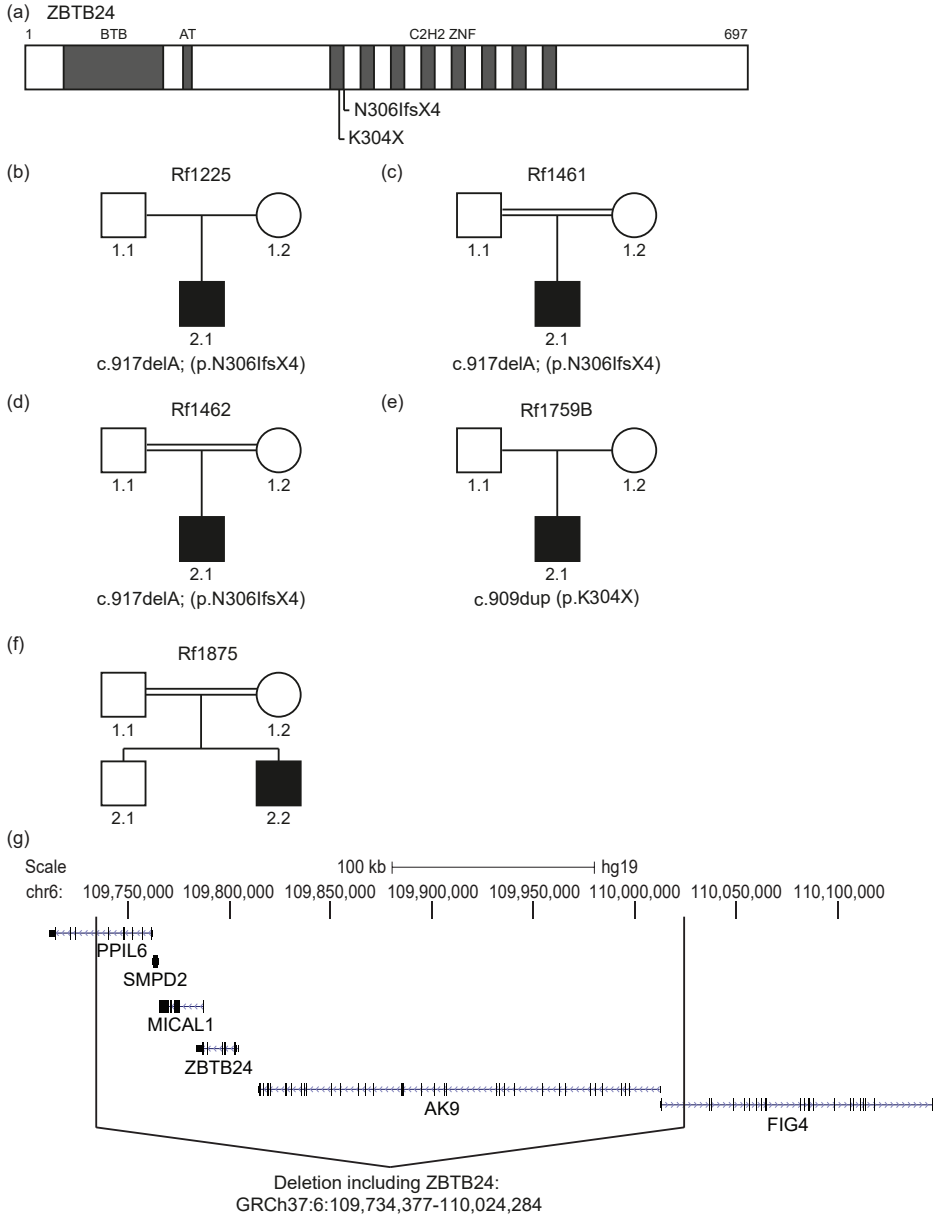


FIGURE 2. ZBTB24 mutations in ICF2 families. **(a)** Schematic representation of the ZBTB24 protein (GenBank: NP_055612.2). The identified amino acid changes of the ICF2 families are indicated. **(b-e)** Overview of the ICF2 pedigrees and their *ZBTB24* mutations. **(f)** Overview of pedigree Rf1875 and **(g)** schematic overview of the deletion on chromosome 6 which was identified in this family. ICF, immunodeficiency, centromeric instability, and facial anomalies.

in which patient Rf1875-2.2 carries a homozygous deletion on chromosome 6 that includes *ZBTB24* and several other genes. Both parents carry this deletion heterozygously, while the genotype of the brother is unknown.

Clinical heterogeneity

The *ZBTB24* mutation c.917delA was found in a total of four ICF2 patients from four unrelated families from Turkey. This suggests that this is a founder mutation. Three of these families are described in this paper, while the fourth family was previously described (P1 in de Greef *et al*. 2011[10] and P49 in Weemaes *et al*. 2013[2]). To assess whether there is clinical heterogeneity among patients carrying an identical mutation, we compared the clinical characteristics of the four male ICF2 patients carrying the c.917delA mutation. We found that the patients from these four families have similar facial anomalies and they all show signs of intellectual disability. In addition, they all have infections, such as bronchopneumonia, however only two (including patient P1 with a reported Candida infection[10]) have opportunistic infections. IgM serum levels are strongly decreased in all of them, whereas IgA levels are strongly decreased in one of them (out of three evaluable cases), and IgG levels are strongly decreased in three of them (except in P1[10]). Finally, B cell numbers are strongly decreased in one, decreased in two, and normal in one (patient P1) of them. They were all treated with IVIG. We also identified two ICF1 patients from one family (Rf1840) which are compound heterozygotes for two missense mutations in *DNMT3B* (c.1750A>G and c.2468G>C). They share similar facial anomalies and bronchopneumonia, however only one of them shows signs of intellectual disability (Rf1840.2.2). They both have strongly decreased IgA levels and normal B-cell numbers. IgM and IgG are strongly decreased in the boy (Rf1840.2.2) and normal and decreased, respectively, in the girl (Rf1840.2.1).

Finally, we identified three ICF1 patients from one family (Rf1771) with the same *DNMT3B* mutation (c.2009G>A). However, two of these patients (twin Rf1771-4.2 and 4.3) were born in 2016, which currently limits the comparison of their phenotypes with that of the third patient (Rf1771-2.1, born in 2013).

Gender bias in ICF2

With the identification of mutations in twelve ICF syndrome cases, a total of 77 genetically confirmed ICF patients has been reported[1; 2; 4; 11; 12; 15; 17-21]. 56% of the ICF syndrome patients carry mutations in *DNMT3B*, 31% carry mutations in *ZBTB24*, 6.5% carry mutations in *CDCA7*, and 6.5% carry mutations in *HELLS* (Fig. 3A). We observed a gender bias in the ICF2 population, with 19 male patients and only 5 female patients out

of 24 patients reported (Fig. 3B). This gender bias is statistically significant (χ^2 (1) = 8.167, p = 0.004) and seems to be specific for ICF2 as it is not observed for the ICF1 cohort or the overall ICF syndrome patient population (Fig. 3B).

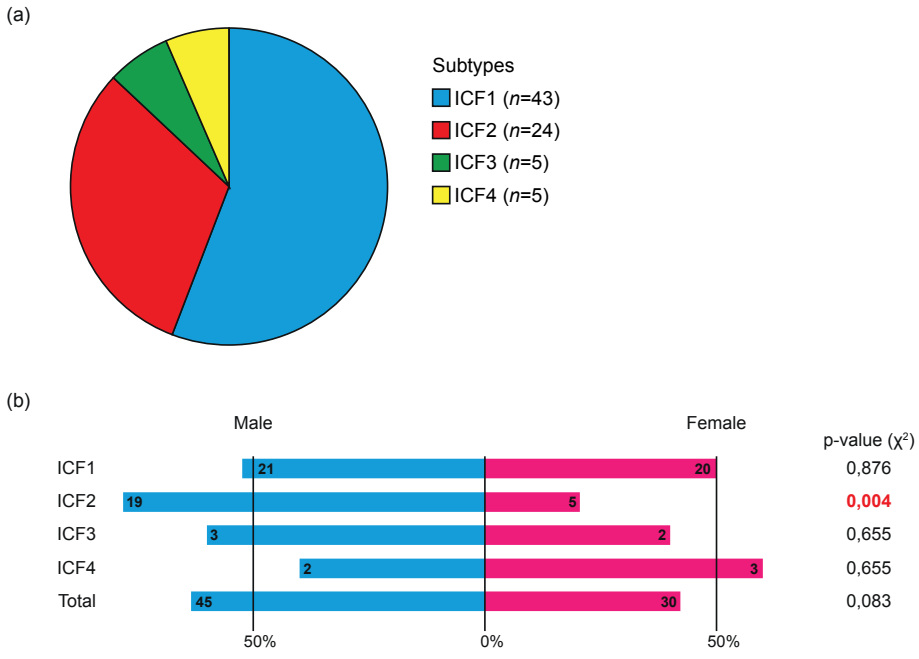


FIGURE 3. Subtype and gender distribution in ICF, immunodeficiency, centromere instability, and facial anomalies (ICF) syndrome. **(a)** Subtype distribution of the ICF patients described in the literature and in this study. **(b)** Gender distribution of the ICF patients described in the literature and in this study. P-values are calculated with the Chi-square test. Out of the 43 reported ICF1 cases, 41 were included in this analysis, 2 were added as missing values. P-values in red reflect a statistically significant difference.

DISCUSSION

In seven ICF1 patients we identified a total of six *DNMT3B* missense mutations, which have not previously been described in ICF1. However, in Rf1840 the arginine residue at postion 823 is replaced by a proline (R823P) and this residue was previously reported to be mutated into a glycine (R823G) in ICF1[19]. All mutations we identified affect highly conserved residues in the catalytic domain of DNMT3B, which is in line with the majority of the previously described ICF1 mutations[2]. Multiple missense mutations

in the catalytic domain have been shown to reduce the methyltransferase activity of DNMT3B[28; 29]. Therefore, we expect that the mutations identified in this study also reduce the catalytic activity of DNMT3B. Three of the newly identified ICF1 patients are from one family and carry the same homozygous missense mutation. Comparison of their clinical phenotypes may facilitate a better understanding of clinical variability at later ages, which is observed in the ICF1 affected siblings from the family Rf1840.

We also identified *ZBTB24* mutations in five previously unreported ICF2 patients. In four ICF2 patients homozygous nonsense mutations in *ZBTB24* were found. Nonsense mutations are the most common type of mutations in ICF2, indicating that most ICF2 patients are expected to completely lack production of ZBTB24 protein. In concordance, we also identified an ICF2 patient (Rf1875) with a homozygous deletion on chromosome 6 that includes *ZBTB24* and several other genes. This indicates that complete absence of ZBTB24 is compatible with human life and supports the conclusion that most ICF2 patients suffer from a ZBTB24 loss of function. In contrast to humans, a Zbtb24 BTB domain deletion in mice, which leads to non-functional Zbtb24 protein, was found to be embryonic lethal[30].

The deletion in ICF2 patient Rf1875-2.2 is not confined to *ZBTB24* but includes *SMPD2*, *MICAL1*, *AK9*, and parts of *PPIL6* and *FIG4*. Most of these genes have not been implicated in disease. PPIL6 is a member of the Cyclophilin Family of Peptidyl-Prolyl Isomerases with unknown activity[31]. *SMPD2* is predicted to encode a sphingomyelin phosphodiesterase, which is able to hydrolyze sphingomyelin *in vitro*, but its *in vivo* function is unclear[32]. MICAL1 is involved in cytoskeleton dynamics via its mono-oxygenase domain that can oxidize target proteins, such as actin, or produce reactive oxygen species[33]. Reduced levels of MICAL1 were detected in the temporal neocortex of patients with temporal lobe epilepsy (TLE) as well as in the hippocampus and temporal lobe of a rat model for TLE after seizures[34]. AK9 is one of the nine adenylate kinases (AK), which regulate adenine nucleotide ratios. Some AK isoforms have been related to human disease, but for *AK9* no relation to disease has been identified[35]. In contrast, mutations in *FIG4* are associated with Charcot-Marie-Tooth disease type 4J (CMT4J)[36], Yunis-Varón syndrome (YVS)[37], amyotrophic lateral sclerosis (ALS)[38], and polymicrogyria[39]. *FIG4* encodes a 5'-phosphoinositide phosphatase involved in endosomal trafficking and autophagy[39]. In patient Rf1875-2.2 the first exon of *FIG4*, which contains the start codon, is deleted, possibly resulting in the absence of full length FIG4 protein in Rf1875-2.2. This patient suffers from epilepsy, which is not a common feature of the ICF syndrome, however was reported in a family where *FIG4* mutations were linked to polymicrogyria. Although none of the other deleted genes have been linked to human disease, we cannot rule out contribution of any of those to the clinical presentation in this patient. Therefore, careful follow up with extra attention on co-morbidities normally not seen in ICF is warranted for this patient.

With the addition of twelve genetically confirmed patients, we re-analyzed the frequencies of the different ICF subtypes. For ICF1 and ICF2 we find similar frequencies, as previously reported[2]. ICF3 and ICF4 are much less frequent than ICF1 and ICF2. Unexpectedly, for ICF2 we found a significant gender bias, with 79% males and only 21% females. This suggests that lacking functional ZBTB24 is more deleterious for females. The mechanistic basis for this difference is currently unclear and warrants further studies focusing on the function of ZBTB24 during (female) development and perhaps X-chromosome inactivation, since ZBTB24 seems to colocalize with heterochromatin[12]. Currently, the limited number of female patients makes it difficult to study phenotype/genotype relations or to compare their phenotypes with those of male ICF2 patients.

In summary, in this report we describe the mutations and clinical characteristics of seven ICF1 cases and five ICF2 cases. We expand the ICF1 mutation spectrum with six new *DNMT3B* mutations and the ICF2 mutation spectrum is extended with a nonsense mutation and a homozygous deletion of chromosome 6, which includes the entire *ZBTB24* locus. For ICF2 we identify a gender bias towards male cases, which, in combination with the embryonic lethality in mice lacking functional Zbtb24, is intriguing and will require follow up studies into the function of ZBTB24.

Acknowledgements

We thank all families for participating in these studies. This study was made possible with financial support from the LUMC van de Kamp fonds, and the Dutch Cancer Society (KWF 2012-5460).

Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

REFERENCES

1. Hagleitner, M.M., Lankester, A., Maraschio, P., Hulten, M., Fryns, J.P., Schuetz, C., Gimelli, G., Davies, E.G., Gennery, A., Belohradsky, B.H., et al. (2008). Clinical spectrum of immunodeficiency, centromeric instability and facial dysmorphism (ICF syndrome). Journal of medical genetics 45, 93-99.
2. Weemaes, C.M., van Tol, M.J., Wang, J., van Ostaijen-ten Dam, M.M., van Eggermond, M.C., Thijssen, P.E., Aytekin, C., Brunetti-Pierri, N., van der Burg, M., Graham Davies, E., et al. (2013). Heterogeneous clinical presentation in ICF syndrome: correlation with underlying gene defects. European journal of human genetics : EJHG 21, 1219-1225.
3. Gennery, A.R., Slatter, M.A., Bredius, R.G., Hagleitner, M.M., Weemaes, C., Cant, A.J., and Lankester, A.C. (2007). Hematopoietic stem cell transplantation corrects the immunologic abnormalities associated with immunodeficiency-centromeric instability-facial dysmorphism syndrome. Pediatrics 120, e1341-1344.
4. Harnisch, E., Buddingh, E.P., Thijssen, P.E., Brooks, A.S., Driessen, G.J., Kersseboom, R., and Lankester, A.C. (2016). Hematopoietic Stem Cell Transplantation in a Patient With ICF2 Syndrome Presenting With EBV-Induced Hemophagocytic Lymphohystiocytosis. Transplantation 100, e35-36.
5. Smeets, D.F., Moog, U., Weemaes, C.M., Vaes-Peeters, G., Merkx, G.F., Niehof, J.P., and Hamers, G. (1994). ICF syndrome: a new case and review of the literature. Hum Genet 94, 240-246.
6. Jeanpierre, M., Turleau, C., Aurias, A., Prieur, M., Ledeist, F., Fischer, A., and Viegas-Pequignot, E. (1993). An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. Human molecular genetics 2, 731-735.
7. Hansen, R.S., Wijmenga, C., Luo, P., Stanek, A.M., Canfield, T.K., Weemaes, C.M., and Gartler, S.M. (1999). The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. ProcNatlAcadSciUSA 96, 14412-14417.
8. Xu, G.L., Bestor, T.H., Bourc'his, D., Hsieh, C.L., Tommerup, N., Bugge, M., Hulten, M., Qu, X., Russo, J.J., and Viegas-Pequignot, E. (1999). Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 402, 187-191.
9. Okano, M., Bell, D.W., Haber, D.A., and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99, 247-257.
10. de Greef, J.C., Wang, J., Balog, J., den Dunnen, J.T., Frants, R.R., Straasheijm, K.R., Aytekin, C., van der Burg, M., Duprez, L., Ferster, A., et al. (2011). Mutations in ZBTB24 are associated with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. American journal of human genetics 88, 796-804.

11. von Bernuth, H., Ravindran, E., Du, H., Frohler, S., Strehl, K., Kramer, N., Issa-Jahns, L., Amulic, B., Ninnemann, O., Xiao, M.S., et al. (2014). Combined immunodeficiency develops with age in Immunodeficiency-centromeric instability-facial anomalies syndrome 2 (ICF2). Orphanet J Rare Dis 9, 116.

12. Nitta, H., Unoki, M., Ichiyanagi, K., Kosho, T., Shigemura, T., Takahashi, H., Velasco, G., Francastel, C., Picard, C., Kubota, T., et al. (2013). Three novel ZBTB24 mutations identified in Japanese and Cape Verdean type 2 ICF syndrome patients. J Hum Genet 58, 455-460.

13. Cerbone, M., Wang, J., Van der Maarel, S.M., D'Amico, A., D'Agostino, A., Romano, A., and Brunetti-Pierri, N. (2012). Immunodeficiency, centromeric instability, facial anomalies (ICF) syndrome, due to ZBTB24 mutations, presenting with large cerebral cyst. Am J Med Genet A 158A, 2043-2046.

14. Chouery, E., Abou-Ghoch, J., Corbani, S., El Ali, N., Korban, R., Salem, N., Castro, C., Klayme, S., Azoury-Abou Rjeily, M., Khoury-Matar, R., et al. (2012). A novel deletion in ZBTB24 in a Lebanese family with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. Clin Genet 82, 489-493.

15. Thijssen, P.E., Ito, Y., Grillo, G., Wang, J., Velasco, G., Nitta, H., Unoki, M., Yoshihara, M., Suyama, M., Sun, Y., et al. (2015). Mutations in CDCA7 and HELLS cause immunodeficiency-centromeric instability-facial anomalies syndrome. Nat Commun 6, 7870.

16. Franceschini, P., Martino, S., Ciocchini, M., Ciuti, E., Vardeu, M.P., Guala, A., Signorile, F., Camerano, P., Franceschini, D., and Tovo, P.A. (1995). Variability of clinical and immunological phenotype in immunodeficiency-centromeric instability-facial anomalies syndrome. Report of two new patients and review of the literature. Eur J Pediatr 154, 840-846.

17. Kutlug, S., Ogur, G., Yilmaz, A., Thijssen, P.E., Abur, U., and Yildiran, A. (2016). Vesicourethral reflux-induced renal failure in a patient with ICF syndrome due to a novel DNMT3B mutation. Am J Med Genet A 170, 3253-3257.

18. Velasco, G., Walton, E.L., Sterlin, D., Hedouin, S., Nitta, H., Ito, Y., Fouyssac, F., Megarbane, A., Sasaki, H., Picard, C., et al. (2014). Germline genes hypomethylation and expression define a molecular signature in peripheral blood of ICF patients: implications for diagnosis and etiology. Orphanet J Rare Dis 9, 56.

19. Ehrlich, M., Buchanan, K.L., Tsien, F., Jiang, G., Sun, B., Uicker, W., Weemaes, C.M., Smeets, D., Sperling, K., Belohradsky, B.H., et al. (2001). DNA methyltransferase 3B mutations linked to the ICF syndrome cause dysregulation of lymphogenesis genes. Human molecular genetics 10, 2917-2931.

20. Rechavi, E., Lev, A., Eyal, E., Barel, O., Kol, N., Barhom, S.F., Pode-Shakked, B., Anikster, Y., Somech, R., and Simon, A.J. (2016). A Novel Mutation in a Critical Region for the Methyl Donor Binding in DNMT3B Causes Immunodeficiency, Centromeric Instability, and Facial Anomalies Syndrome (ICF). J Clin Immunol 36, 801-809.

21. Jiang, Y.L., Rigolet, M., Bourc'his, D., Nigon, F., Bokesoy, I., Fryns, J.P., Hulten, M., Jonveaux, P., Maraschio, P., Megarbane, A., et al. (2005). DNMT3B mutations and DNA methylation defect define two types of ICF syndrome. Hum Mutat 25, 56-63.

22. van den Boogaard, M.L., Lemmers, R.J., Balog, J., Wohlgemuth, M., Auranen, M., Mitsuhashi, S., van der Vliet, P.J., Straasheijm, K.R., van den Akker, R.F., Kriek, M., et al. (2016). Mutations in DNMT3B Modify Epigenetic Repression of the D4Z4 Repeat and the Penetrance of Facioscapulohumeral Dystrophy. American journal of human genetics 98, 1020-1029.

23. Ng, P.C., and Henikoff, S. (2001). Predicting deleterious amino acid substitutions. Genome Res 11, 863-874.

24. Schwarz, J.M., Cooper, D.N., Schuelke, M., and Seelow, D. (2014). MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 11, 361-362.

25. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. Nat Methods 7, 248-249.

26. Choi, Y., Sims, G.E., Murphy, S., Miller, J.R., and Chan, A.P. (2012). Predicting the functional effect of amino acid substitutions and indels. PloS one 7, e46688.

27. Tauris, P., and Hansen, P.W. (1983). Functional studies on lymphocytes from two siblings with congenital hypogammaglobulinaemia. Scand J Haematol 30, 117-124.

28. Gowher, H., and Jeltsch, A. (2002). Molecular enzymology of the catalytic domains of the Dnmt3a and Dnmt3b DNA methyltransferases. J Biol Chem 277, 20409-20414.

29. Moarefi, A.H., and Chedin, F. (2011). ICF syndrome mutations cause a broad spectrum of biochemical defects in DNMT3B-mediated de novo DNA methylation. J Mol Biol 409, 758-772.

30. Wu, H., Thijssen, P.E., de Klerk, E., Vonk, K.K., Wang, J., den Hamer, B., Aytekin, C., van der Maarel, S.M., and Daxinger, L. (2016). Converging disease genes in ICF syndrome: ZBTB24 controls expression of CDCA7 in mammals. Human molecular genetics.

31. Davis, T.L., Walker, J.R., Campagna-Slater, V., Finerty, P.J., Paramanathan, R., Bernstein, G., MacKenzie, F., Tempel, W., Ouyang, H., Lee, W.H., et al. (2010). Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. PLoS Biol 8, e1000439.

32. Horres, C.R., and Hannun, Y.A. (2012). The roles of neutral sphingomyelinases in neurological pathologies. Neurochem Res 37, 1137-1149.

33. Giridharan, S.S., and Caplan, S. (2014). MICAL-family proteins: Complex regulators of the actin cytoskeleton. Antioxid Redox Signal 20, 2059-2073.

34. Luo, J., Xu, Y., Zhu, Q., Zhao, F., Zhang, Y., Peng, X., Wang, W., and Wang, X. (2011). Expression pattern of Mical-1 in the temporal neocortex of patients with intractable temporal epilepsy and pilocarpine-induced rat model. Synapse 65, 1213-1221.

35. Panayiotou, C., Solaroli, N., and Karlsson, A. (2014). The many isoforms of human adenylate kinases. Int J Biochem Cell Biol 49, 75-83.

36. Chow, C.Y., Zhang, Y., Dowling, J.J., Jin, N., Adamska, M., Shiga, K., Szigeti, K., Shy, M.E., Li, J., Zhang, X., et al. (2007). Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. Nature 448, 68-72.

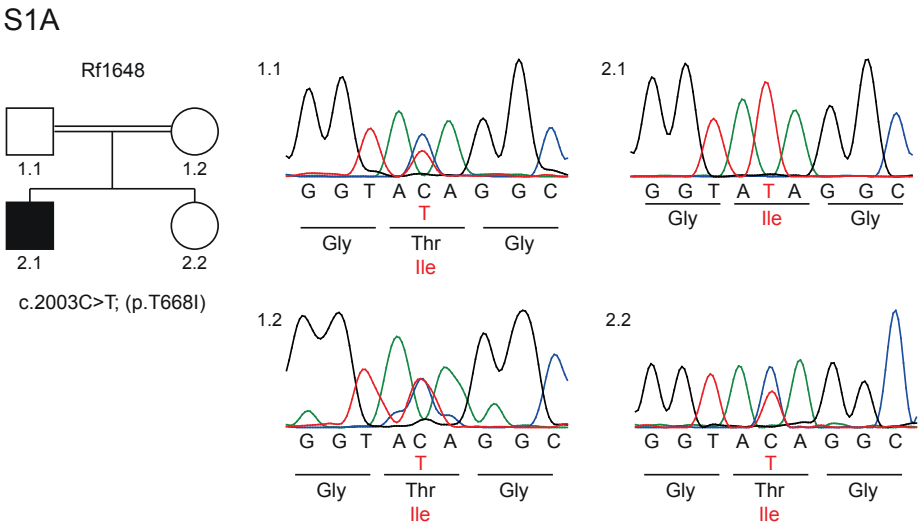
37. Campeau, P.M., Lenk, G.M., Lu, J.T., Bae, Y., Burrage, L., Turnpenny, P., Roman Corona-Rivera, J., Morandi, L., Mora, M., Reutter, H., et al. (2013). Yunis-Varon syndrome is caused by mutations in FIG4, encoding a phosphoinositide phosphatase. American journal of human genetics 92, 781-791.

38. Chow, C.Y., Landers, J.E., Bergren, S.K., Sapp, P.C., Grant, A.E., Jones, J.M., Everett, L., Lenk, G.M., McKenna-Yasek, D.M., Weisman, L.S., et al. (2009). Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. American journal of human genetics 84, 85-88.

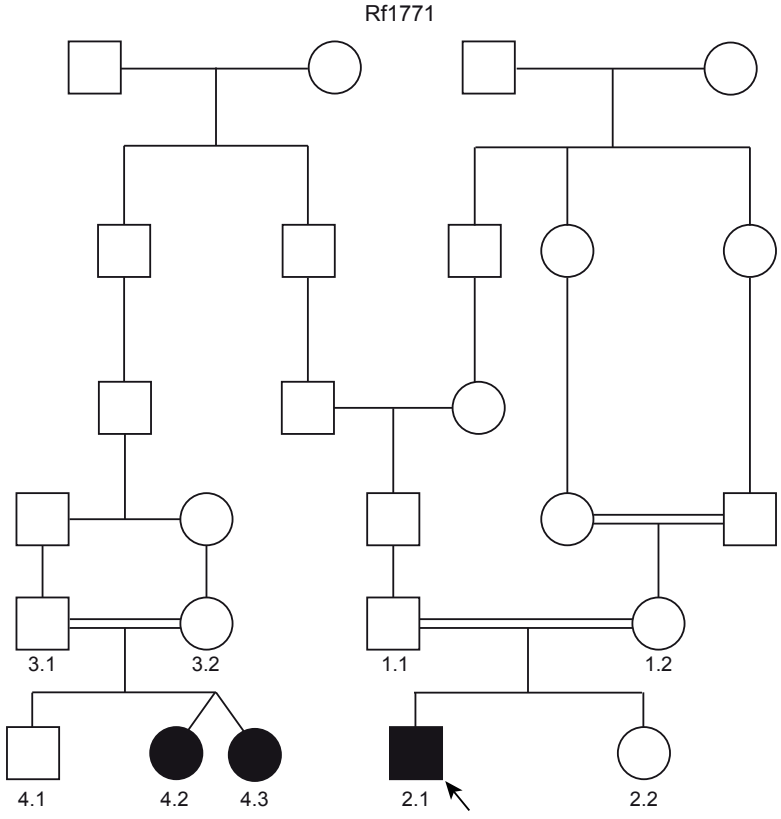
39. Baulac, S., Lenk, G.M., Dufresnois, B., Ouled Amar Bencheikh, B., Couarch, P., Renard, J., Larson, P.A., Ferguson, C.J., Noe, E., Poirier, K., et al. (2014). Role of the phosphoinositide phosphatase FIG4 gene in familial epilepsy with polymicrogyria. Neurology 82, 1068-1075.

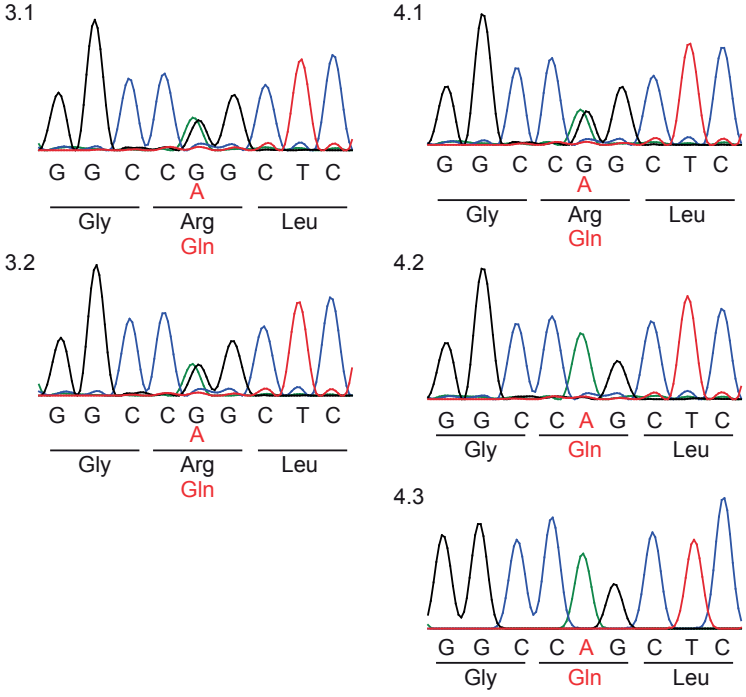
SUPPLEMENTARY INFORMATION

Supplementary figures

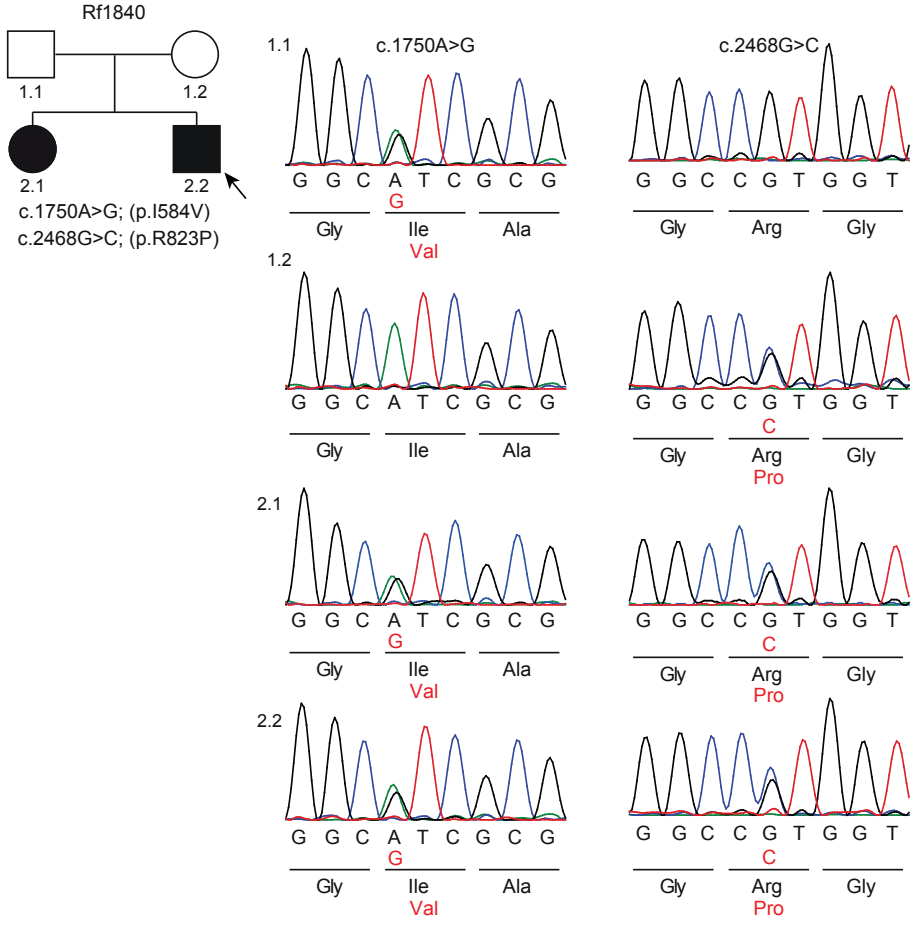


S1B

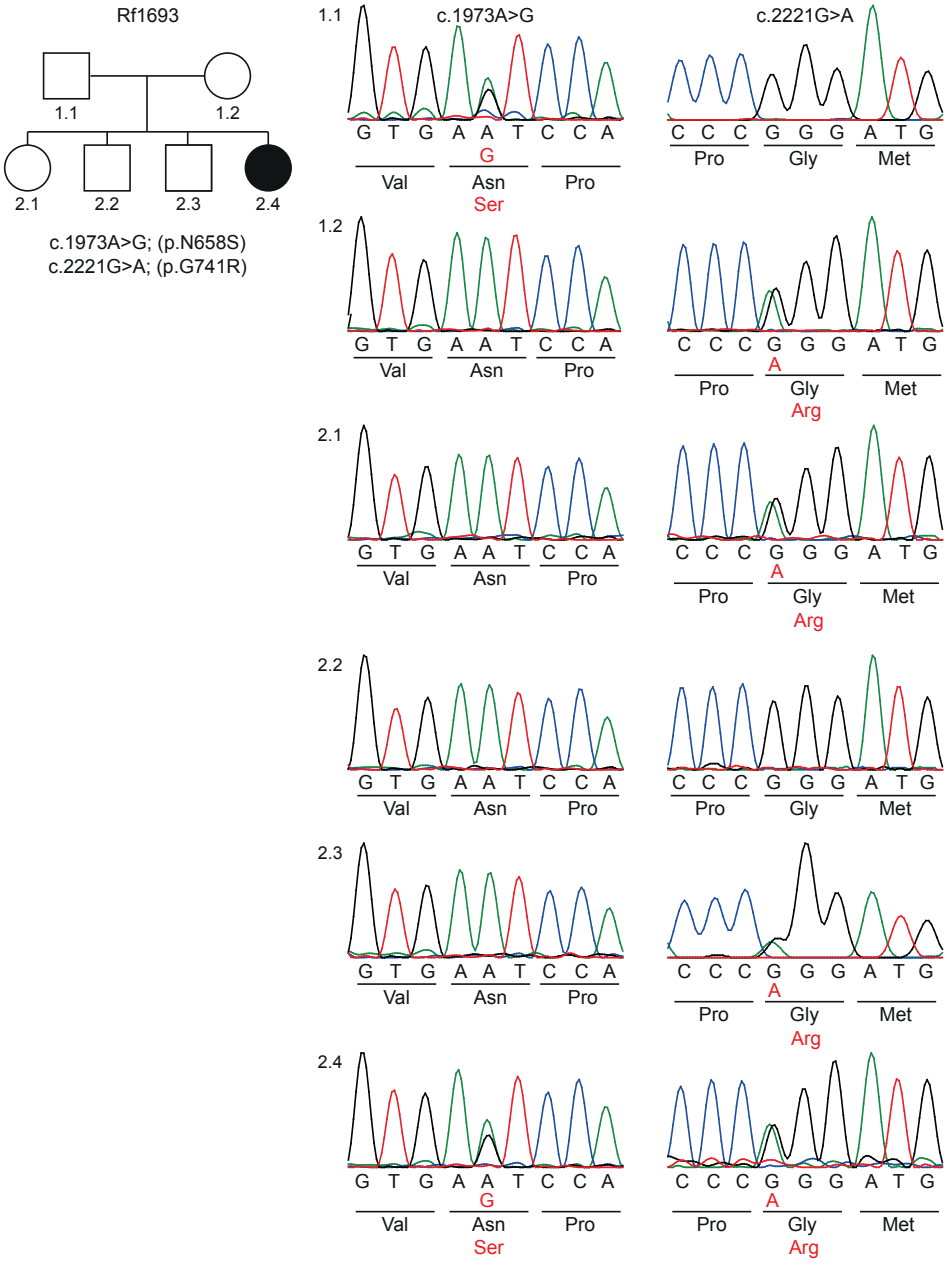

Rf1771
3.1
3.2
1.1
1.2
4.1
4.2
4.3
2.1
2.2
c.2009G>A; (p.R670Q)
1.1
G G C C G G C T C
A
Gly
Arg
Gln
Leu
2.1
G G C C A G C T C
Gly
Gln
Leu
1.2
G G C C G G C T C
A
Gly
Arg
Gln
Leu
2.2
G G C C G G C T C
A
Gly
Arg
Gln
Leu


3.1
G G C C G G C T C
A
Gly
Arg
Gln
Leu
3.2
G G C C G G C T C
A
Gly
Arg
Gln
Leu
4.1
G G C C G G C T C
A
Gly
Arg
Gln
Leu
4.2
G G C C A G C T C
Gly
Gln
Leu
4.3
G G C C A G C T C
Gly
Gln
Leu

S1C

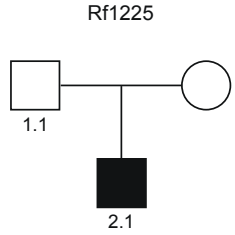

Rf1840
1.1
1.2
2.1
2.2
c.1750A>G; (p.I584V)
c.2468G>C; (p.R823P)
1.1
c.1750A>G
c.2468G>C
G G C A T C G C G
G
Gly Ile Ala
Val
G G C C G T G G T
Gly Arg Gly
1.2
G G C A T C G C G
Gly Ile Ala
G G C C G T G G T
C
Gly Arg Gly
Pro
2.1
G G C A T C G C G
G
Gly Ile Ala
Val
G G C C G T G G T
C
Gly Arg Gly
Pro
2.2
G G C A T C G C G
G
Gly Ile Ala
Val
G G C C G T G G T
C
Gly Arg Gly
Pro

S1D

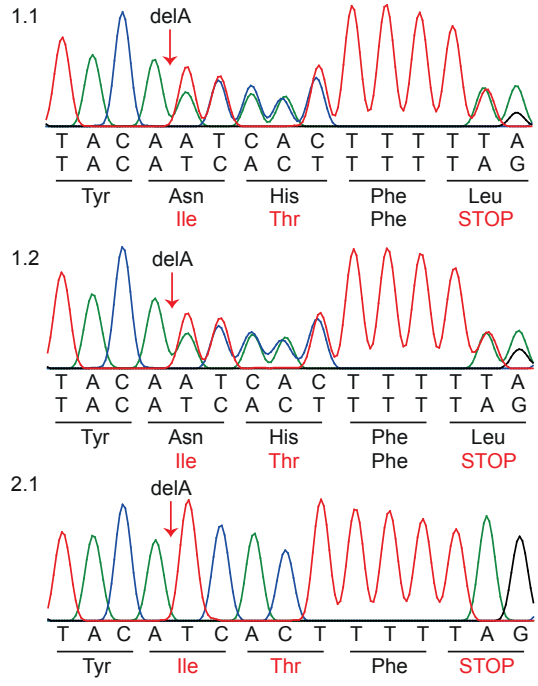


SUPPLEMENTARY FIGURE S1. *DNMT3B* mutations in ICF1 families. **(a-d)** Overview of the ICF1 pedigrees, their DNMT3B mutations and the Sanger sequence track of these mutations. For families with multiple ICF patients, probands are indicated with an arrow.

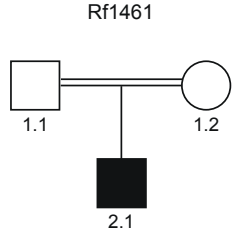
S2A



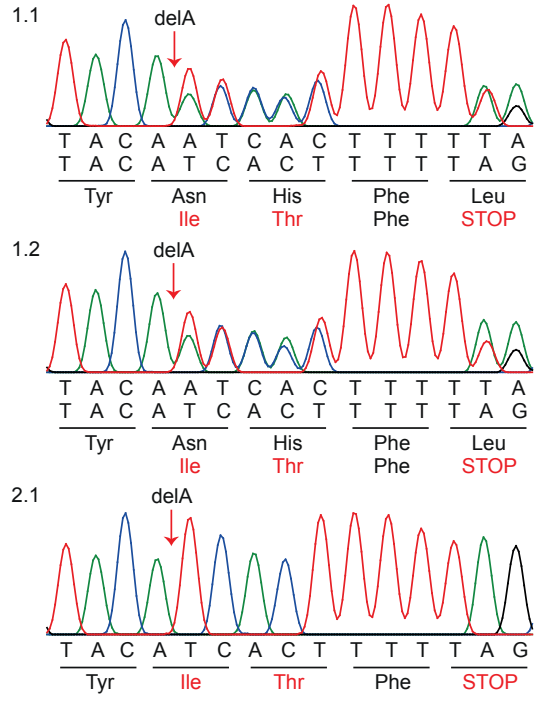
c.917delA; (p.N306IfsX4)

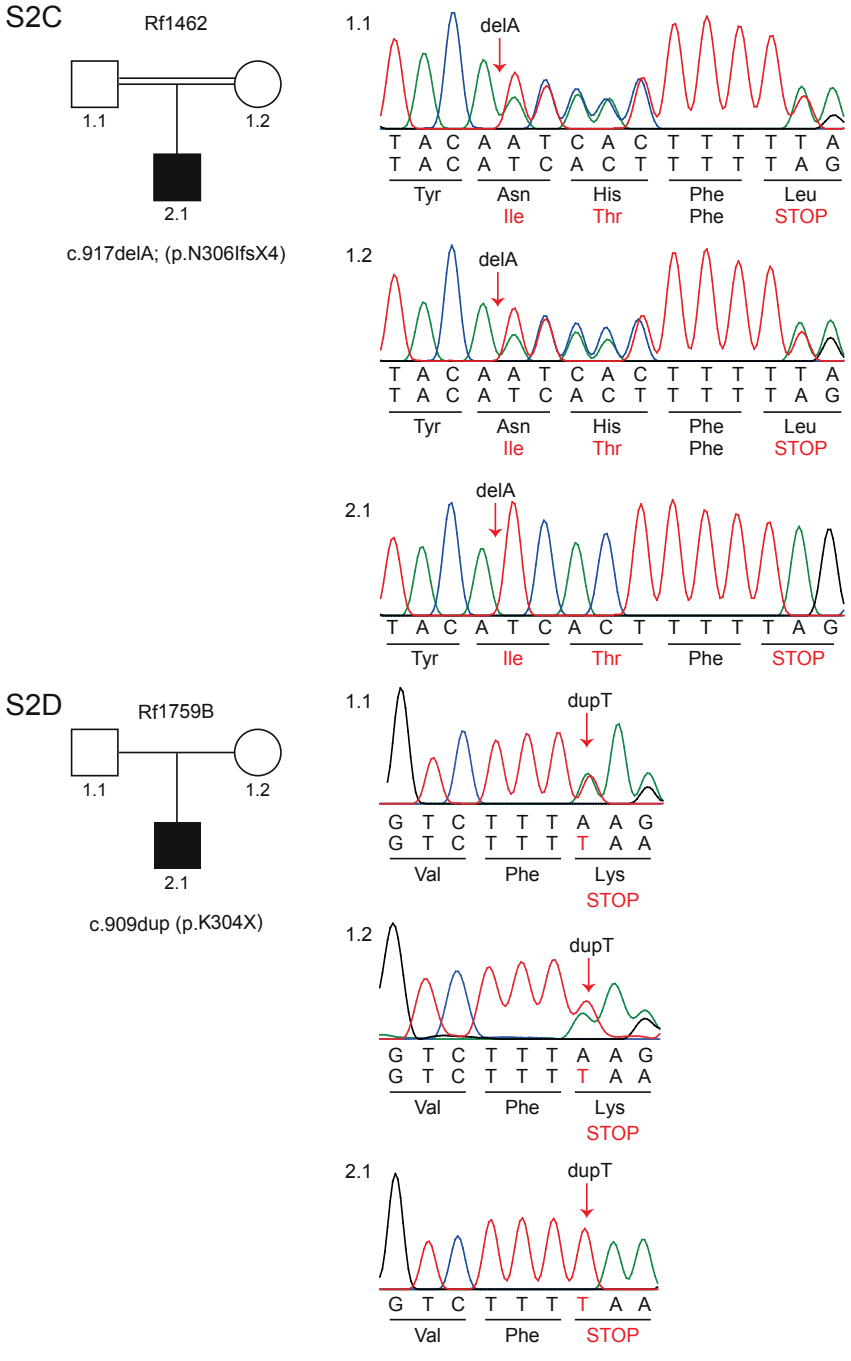


S2B



c.917delA; (p.N306IfsX4)





SUPPLEMENTARY FIGURE S2. *ZBTB24* mutations in ICF2 families. **(a-d)** Overview of the ICF2 pedigrees, their *ZBTB24* mutations and the Sanger sequence track of these mutations.

Supplementary tables

SUPPLEMENTARY TABLE 1. Overview of the clinical data of the ICF patients.

	ICF1								
Patient	**Rf1648-2.1**	**Rf1771-2.1**	**Rf1771-4.2**	**Rf1771-4.3**	**Rf1840-2.1**	**Rf1840-2.2**	**Rf1693-2.4**	**Total this study**	**Weemaes et al. EJHG 2013**
Year of birth	2004	2013	2016	2016	2007	2009	2009		
Gene defect	DNMT3B	DNMT3B	DNMT3B	DNMT3B	DNMT3B	DNMT3B	DNMT3B	DNMT3B	DNMT3B
Mutation Paternal Allele	c.2003C>T	c.2009G>A	c.2009G>A	c.2009G>A	c.1705A>G	c.1705A>G	c.1973A>G		
Mutation Maternal Allele	c.2003C>T	c.2009G>A	c.2009G>A	c.2009G>A	c.2468G>C	c.2468G>C	c.2221G>A		
Patients included								7	23
Facial anomalies								7/7	21/22
hypertelorism	+	+			+	+	-	4/5	14/18
flat nasal bridge	-	+	+	+	+	+	+	6/7	13/16
epicanthus	+	-	+	+	+	+	+	6/7	14/17
up-turned nose	+	-	+	+	+	+	-	5/7	6/9
macroglossia	-	-	-	-		-	-	0/6	5/11
telecanthus	-	-	-	-		+	+	2/6	3/11
micrognathia	+	-	+	+		-	+	4/6	5/12
low-set ears	+	+	-	-	+	+	-	4/7	6/14
round face	+	+	+	+	+	+	+	7/7	8/10
Growth and development									
gestional age < 37 weeks	-	-	-	-	-	-	-	0/7	3/20
birth weight < P10	+		-	-	-	+	-	2/6	9/20
failure to thrive	-	-	+	-	-	+	-	2/7	8
delay in motor development	-	-			-	+		1/4	9/16
delay in speech development	+				-	+		2/3	14/16
Malformations									
congenital			+					1	7
cerebral									2
Intelligence									
normal		+			+			2/5	11/20
retardation	+					+	+	3/5	9/20
Neurology									
seizures								0	3
Gastrointestinal problems									
diarrhea	+							1	7/14
Infections									
otitis	+							1	8/13
bronchopneumonia	+	+	+	+	+	+	-	6/7	16/16
sepsis									5
meningitis							+*	1	
Candida									4
Pneumocystis jirovecii									2
cytomegalovirus	-	+	-	-	-	-	-	1	2
Malignancy									2

The gene defects and clinical data of the ICF patients described in this study are listed per patient. Columns 9 and 16 list the total number of patients displaying the respective trait/total number of patients of which data on the respective trait is reported. As a comparison the same data are listed from an earlier study in column 10 and 17 [1]. *: Pseudomonas, **: Streptococcus pneumoniae

	ICF2						
	Rf1225-2.1	**Rf1461-2.1**	**Rf1462-2.1**	**Rf1759B-2.1**	**Rf1875-2.2**	**Total this study**	**Weemaes et al. EJHG 2013**
	2011	2012	2001	1985	1995		
	ZBTB24	ZBTB24	ZBTB24	ZBTB24	ZBTB24	ZBTB24	ZBTB24
	c.917delA	c.917delA	c.917delA	c.909dup	Deletion		
	c.917delA	c.917delA	c.917delA	c.909dup	Deletion		
						5	13
						5/5	13/13
	+	+	+	+	+	5/5	7/13
	+	+	+	+	+	5/5	8/9
	+	+	+	+	-	4/5	7/8
	+	+	+	-		3/4	4/7
	+	-	+	-		2/4	1/5
	+	+	+	+	-	4/5	2/4
	+	+	-	-	+	3/5	3/8
	+	+	+		+	4/4	5/7
	+	+	+	-		3/4	6/8
	+	-	-	+	-	2/5	1/7
	+	+	+	+	-	4/5	4/6
	-	-	-	-	-	0/5	3
	-	+	+	-	+	3/5	7/8
	+	+	+	+	+	5/5	11/13
							2
							2
				+		1/5	0/13
	+	+	+		+	4/5	13/13
	-	-	-	+	+	2	1
	-	-	-	-	-	0	2/6
				+		1	2/6
	+	+	+	+	+	5/5	5/7
							1
			+**		+**	2	
	+					1	2
	+					1	2
	+	-	-	-	-	1	
							1

SUPPLEMENTARY TABLE 2. Serum levels of the different immunoglobulin isotypes and numbers of peripheral blood B cells at first analysis.

	ICF1									ICF2						
	Rf1648-2.1	Rf1771-2.1	Rf1771-4.2	Rf1771-4.3	Rf1840-2.1	Rf1840-2.2	Rf1693-2.4	This study	Weemaes et al. EJHG 2013	Rf1225-2.1	Rf1461-2.1	Rf1462-2.1	Rf1759B-2.1	Rf1875-2.2	This study	Weemaes et al. EJHG 2013
Patients included	x	x	x	x	x	x	x	7	23	x	x	x	x	x	5	13
IgG																
Patients analyzed	x	x	x	x	x	x	x	7	13	x	x	x	x	x	5	12
Normal								0	1						0	2
Decreased		x				x		2	1				x		1	4
Strongly decreased	x				x		x	3	11	x	x	x		x	4	6
Inconclusive*			x	x				2	0						0	0
IgA																
Patients analyzed	x	x	x	x	x	x	x	7	19	x	x	x	x	x	5	13
Normal								0	0				x		1	5
Decreased		x						1	0			x			1	0
Strongly decreased	x				x	x		3	13	x				x	2	4
Inconclusive*			x	x			x	3	6		x				1	4
IgM																
Patients analyzed	x	x	x	x	x	x	x	7	19	x	x	x	x	x	5	13
Normal						x		1	3						0	1
Decreased								0	0				x	x	2	3
Strongly decreased	x	x	x	x	x		x	6	16	x	x	x			3	9
B-cells																
Patients analyzed	x	x	x	x	x	x	x	7	9	x	x	x	x	x	5	8
Normal	x		x	x	x	x	x	6	4				x		1	7
Decreased		x						1	2	x	x				2	0
Strongly decreased								0	3			x		x	2	1

The serum immunoglobulin levels of the ICF patients described in this study are categorized as normal, decreased and strongly decreased for each isotype using reference values for age-matched healthy controls according to Kanariou *et al.,* 1995[2]. Peripheral blood B-cell numbers are categorized as normal, decreased and strongly decreased based on reference values for age-matched healthy controls according to Comans-Bitter *et al.*, 1997 [3]. As a comparison, the same data are listed from an earlier study [1]. For some patients from this earlier study IgG data is not included because of IVIG treatment already initiated at time of first analysis. In other cases data are lacking because they were not included at first analysis.

*: Inconclusive: measured IgA concentrations as well as lower threshold for normal range in healthy age-matched controls are at or below the sensitivity level of the quantitative assay applied; two ICF1 cases were diagnosed at 4 months of age and the measured decreased IgG levels might be from maternal origin.

SUPPLEMENTARY TABLE 3. Variant predictions for identified *DNMT3B* missense variants

	c.2003C>T, (p.T668I)	**c.1750A>G, (p.I584V)**	**c.2468G>C, (p.R823P)**
SIFT	Deleterious (score: 0)	Deleterious (score: 0)	Deleterious (score: 0.01)
Mutation Taster	Disease causing (p-value: 1)	Disease causing (p-value: 0.995)	Disease causing (p-value: 1)
PolyPhen-2 HumVar	Probably damaging (score: 0.984)	Possibly damaging (score: 0.610)	Benign (score: 0.180)
PROVEAN	Deleterious (score: -5.692)	Neutral (score: -0.854)	Deleterious (score: -6.419)
	c.1973A>G, (p.N658S)	**c.2221G>A, (G741R)**	**c.2009G>A, p.(R670Q)**
SIFT	Tolerated (score: 0.16)	Deleterious (score: 0.02)	Deleterious (score: 0)
Mutation Taster	Disease causing (p-value: 0.924)	Disease causing (p-value: 1)	Disease causing (p-value: 1)
PolyPhen-2 HumVar	Benign (score: 0.266)	Probably damaging (score: 0.993)	Possibly damaging (score: 0.630)
PROVEAN	Deleterious (score: -4.638)	Deleterious (score: -7.378)	Deleterious (score: -3.817)

REFERENCES

1. Weemaes, C.M., van Tol, M.J., Wang, J., van Ostaijen-ten Dam, M.M., van Eggermond, M.C., Thijssen, P.E., Aytekin, C., Brunetti-Pierri, N., van der Burg, M., Graham Davies, E., et al. (2013). Heterogeneous clinical presentation in ICF syndrome: correlation with underlying gene defects. European journal of human genetics : EJHG 21, 1219-1225.
2. Kanariou, M., Petridou, E., Liatsis, M., Revinthi, K., Mandalenaki-Lambrou, K., and Trichopoulos, D. (1995). Age patterns of immunoglobulins G, A & M in healthy children and the influence of breast feeding and vaccination status. Pediatr Allergy Immunol 6, 24-29.
3. Comans-Bitter, W.M., de Groot, R., van den Beemd, R., Neijens, H.J., Hop, W.C., Groeneveld, K., Hooijkaas, H., and van Dongen, J.J. (1997). Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. The Journal of pediatrics 130, 388-393.