

Supplemental Data

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**Mutations in *ZBTB24* Are Associated
with Immunodeficiency, Centromeric Instability,
and Facial Anomalies Syndrome Type 2**

**Jessica C. de Greef, Jun Wang, Judit Balog, Johan T. den Dunnen, Rune R. Frants,
Kirsten R. Straasheijm, Caner Aytakin, Mirjam van der Burg, Laurence Duprez, Alina
Ferster, Andrew R. Gennery, Giorgio Gimelli, Ismail Reisli, Catharina Schuetz, Ansgar
Schulz, Dominique F.C.M. Smeets, Yves Sznajer, Cisca Wijmenga, Marja C. van
Eggermond, Monique M. van Ostaijen-ten Dam, Arjan C. Lankester, Maarten J.D. van
Tol, Peter J. van den Elsen, Corry M. Weemaes, and Silvère M. van der Maarel**

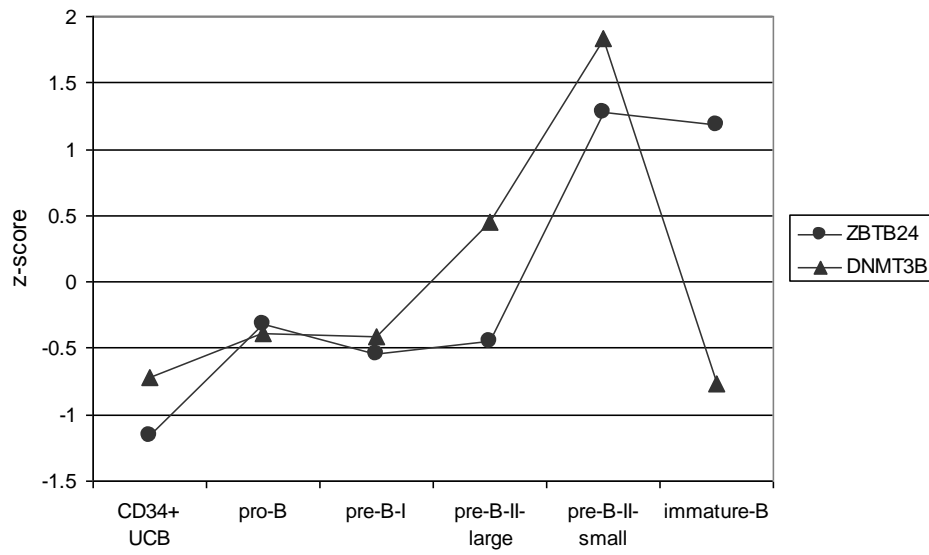


Figure S1. Expression Pattern of *ZBTB24* and *DNMT3B* during Bone Marrow Precursor B-Cell Differentiation

Expression levels were determined by gene expression profiling using Affymetrix HG-U133 set GeneChip arrays.¹ The expression values of the probe sets are normalized to zero mean and unit standard deviation (z-score).

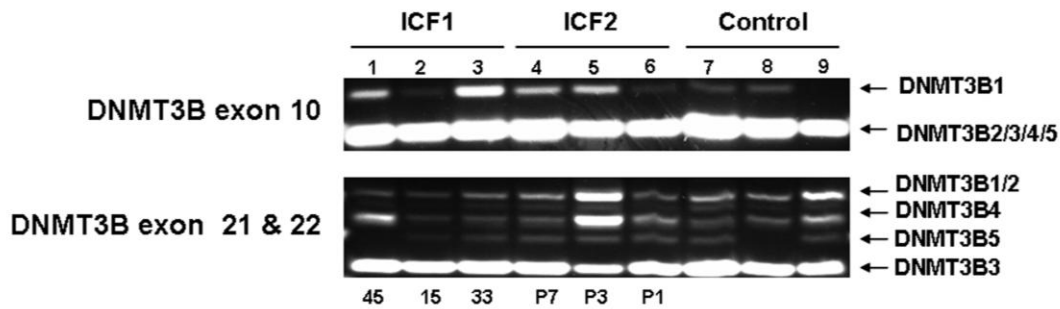
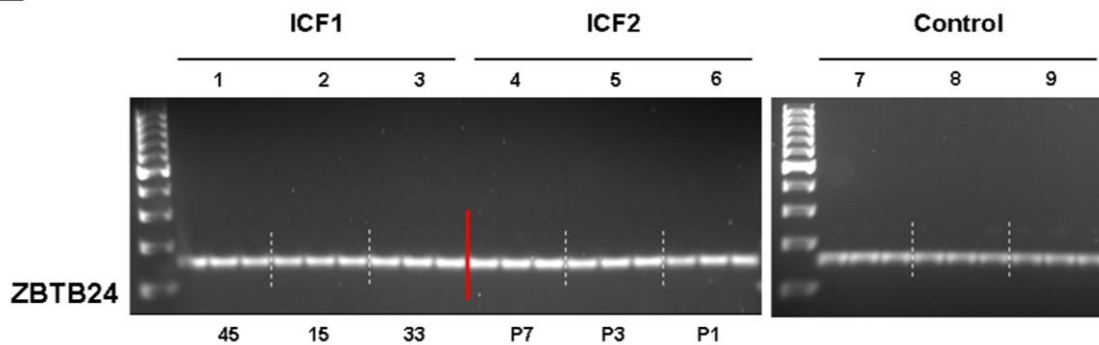
A**B**

Figure S2. Expression of *DNMT3B* Splice Variants and *ZBTB24* in ICF1 and ICF2 Patients and Control Individuals

RNA was isolated from EBV-immortalized B cells (samples 2-4 and 6-9), fibroblasts (sample 1) and leukocytes (sample 5). Samples 1, 2 and 3 were described before in a large study as patients 45, 15 and 33, respectively.² Sample 4 is P7, sample 5 is P3 and sample 6 is P1.

(A) Expression of five major *DNMT3B* splice variants. RT-PCR was performed with primer pairs recognizing exon 10 or exons 21 and 22. *DNMT3B1* is the full-length RNA product. The *DNMT3B2* transcript lacks exon 10. The *DNMT3B3* transcript lacks exons 10, 21 and 22. The *DNMT3B4* transcript lacks exons 10 and 21. Finally, the *DNMT3B5* transcript lacks exons 10 and 22.^{3,4}

(B) *ZBTB24* expression (experiment performed in triplicate). RT-PCR was performed with a primer pair recognizing the full-length RNA transcript.

Table S1. Primer Sequences for *ZBTB24* Mutation Analysis

Primer name	Primer sequence	<i>ZBTB24</i> exon
Exon 2.1_forward	5'-GGGCTGGCATTAAACCTTTTA-3'	exon 2 (ATG)
Exon 2.1_reverse	5'-TTCTTCCTCTGCAGCCAGTT-3'	exon 2 (ATG)
Exon 2.2_forward	5'-GTGCCCCAGTGGTTGTTATC-3'	exon 2
Exon 2.2_reverse	5'-ACAATGCCCATCACACAGAA-3'	exon 2
Exon 3_forward	5'-TCATTGTTGGCCTTTTGGAT-3'	exon 3
Exon 3_reverse	5'-CAATGACACCACGTTGGAAA-3'	exon 3
Exon 4_forward	5'-GTAGAAAATGGGGACCTGGA-3'	exon 4
Exon 4_reverse	5'-ATGGTGGCGGACAATAACAT-3'	exon 4
Exon 5_forward	5'-TCAGGGAAGTGCTTTAACACAA-3'	exon 5
Exon 5_reverse	5'-TTCCAAACACAGGCTTAGCA-3'	exon 5
Exon 6_forward	5'-TTGTGAAACCGAAGTGTTTTT-3'	exon 6
Exon 6_reverse	5'-TGCACACAAAATTCTGCAAAG-3'	exon 6
Exon 7_forward	5'-TCTTGATTGCTTCCCCCTAA-3'	exon 7 (TGA)
Exon 7_reverse	5'-GAGCCTGATTTCAAGCGTTC-3'	exon 7 (TGA)

Table S2. Clinical Characteristics of ICF2 Patients with *ZBTB24* Mutations Compared with the Clinical Characteristics of ≤20 ICF1

Patients with *DNMT3B* Mutations

	P1	P2	P3	P5	P6	P7	P10	ICF1 (n≤20)
ZBTB24_allele 1	p.Asn306IlefsX4 (c.917delA)	p.Ser16X (c.47C>G)	p.Arg320X (c.958C>T)	p.Val168SerfsX28 (c.501dup)	p.Ser278X (c.833C>G)	p.Ser278X (c.833C>G)	p.Arg457X (c.1369C>T)	x
ZBTB24_allele 2	p.Asn306IlefsX4 (c.917delA)	p.Ser16X (c.47C>G)	p.Arg320X (c.958C>T)	p.Val168SerfsX28 (c.501dup)	p.Cys408Gly (c.1222T>G)	p.Cys408Gly (c.1222T>G)	p.Arg457X (c.1369C>T)	x
Sex	M	F	F	M	M	F	M	x
Birth year	2006	1987	1983	1997	1998	2001	1981	x
Age at death (years)	-	13	11	-	4	-	-	x
Origin	Turkish	Scottish	Turkish	Turkish	German	German	Italian	x
Birth weight (grams)	NA	1300	NA	2340	NA	2270	2950	<2950: 6/17
Motor development delay	yes	yes	yes	NA	NA	yes	no	10/17
Mental retardation	yes	yes	yes	yes	yes	yes	yes	9/20
Facial anomalies	yes	yes	yes	yes	yes	yes	yes	18/18
Gastrointestinal problems	diarrhea	-	reflux	diarrhea	-	-	-	diarrhea: 7/16, malabsorption: 2/16
Infections	Arthritis, bronchopneumonia, Candida, gastroenteritis, meningitidis, otitis	Bronchopneumonia, Candida, Pneumocystis jirovecii	Bronchopneumonia, sepsis	Bronchopneumonia, Pneumocystis jirovecii	Bronchopneumonia, otitis	Bronchopneumonia	Bronchopneumonia	Bronchopneumonia: 8/18, Candida: 2/18, otitis: 2/18, Pneumocystis jirovecii: 2/18, sepsis: 2/18,

IgG (g/L)	1.15 (2.0-6.7)	1.50 (4.1-11.0)	<2.00 (4.1-11.0)	1.50 (4.0-11.0)	<2.00 (4.1-11.0)	3.00 (4.0-11.0)	3.80 (7.0-16.0)	Hypogammaglobulinemia: 19/20
IgA (g/L)	<0.06 (0.08-0.7)	0.07 (0.1-1.6)	0.00 (0.1-1.6)	0.07 (0.1-1.6)	2.89 (0.1-1.6)	0.81 (0.1-1.6)	0.20 (0.7-4.0)	IgA deficiency: 1/20
IgM (g/L)	<0.04 (0.3-1.0)	0.22 (0.5-1.8)	0.00 (0.5-1.8)	<0.02 (0.5-1.8)	0.03 (0.5-1.8)	0.23 (0.5-1.8)	1.24 (0.4-2.3)	IgM deficiency: 0/20
CD3 T-cells (counts/μl)	5060 (2400-6900)	1440 (900-4500)	1140 (900-4500)	4070 (1600-6700)	2150 (900-4500)	6200 (2400-6900)	720 (800-3500)	Decreased: 0/12
CD4 T-cell subset (counts/μl)	4420 (1400-5100)	860 (500-2400)	990 (500-2400)	2860 (1000-4600)	1620 (500-2400)	4530 (1400-5100)	270 (400-2100)	Decreased: 0/12
CD8 T-cell subset (counts/μl)	630 (600-2200)	580 (300-1600)	240 (300-1600)	1210 (400-2100)	440 (300-1600)	1260 (600-2200)	500 (200-1200)	Decreased: 1/12
CD19 B-cells (counts/μl)	1140 (700-2500)	770 (200-2100)	160 (200-2100)	990 (600-2700)	660 (200-2100)	1840 (700-2500)	50 (200-600)	Decreased:4/8

NA: not analyzed. Between brackets: Ig reference values⁵ and reference values lymphocyte (sub)populations.⁶

Table S3. Primer Sequences for *DNMT3B* and *ZBTB24* RT-PCR Analysis

Primer name	Primer sequence
ZBTB24_forward	5'-GCCGGATTTAACCCTTAGTG-3'
ZBTB24_reverse	5'-GAGCCTGATTTCAAGCGTTC-3'
DNMT3B ex10_forward	5'-AGCCCATGTTGGAGTGGGCCACG-3'
DNMT3B ex10_reverse	5'-CATCCCCTCGGTCTTTGCCGTTGTTAT-3'
DNMT3B ex21/22_forward	5'-CCTGCTGAATTACTCACGCCCC-3'
DNMT3B ex21/22_reverse	5'-GTCTGTGTAGTGCACAGGAAAGCC-3'
GAPDH_forward	5'-GAGTCAACGGATTTGGTCGT-3'
GAPDH_reverse	5'-TTGATTTTGGAGGGATCTCG-3'
GUSB_forward	5'-CTCATTTGGAATTTTGCCGATT-3'
GUSB_reverse	5'-CCGAGTGAAGATCCCCTTTTTA-3'
ACTB_forward	5'-GGAGTCCTGTGGCATCCACG-3'
ACTB_reverse	5'-CTAGAAGCATTGCGGTGGA-3'

Supplemental References

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