Supplementary Table 1. Clinical features in 15 patients at presentation.

Clinical Feature	Family A (14 patients)	Family B (1 patient)
Sinopulmonary infections	11/14 (78.6%)	+
Chronic diarrhea	10/14 (71.4%)	+
Sepsis	5/14 (35.7%)	+
Failure to thrive	4/14 (28.6%)	+
Conjunctivitis	3/14 (21.4%)	–
Viral meningitis	2/14 (14.3%)	–
Thrush	1/14 (7.1%)	–
Gluteal abscess	1/14 (7.1%)	–

Supplementary Table 2. Patients' hemogram and iron studies.

Patient	A1	A2	A3	B1
Age	6 years	14 months	4 months	5 years
Hemogram				
Platelets, cells/mL x10^3	***86*** (227 – 350)	***74*** (185 – 399)	***47*** (147 – 423)	***55*** (211 – 370)
Hemoglobin (g/dL)	11.1 (11 – 13.3)	***10.1*** (10.4 – 12.5)	11.7 (10.7 – 13.4)	***9.5*** (11.0 – 12.8)
MCV (fL)	***77.1*** (78.2 – 83.9)	***69.4*** (75.6 – 83.1)	***72.3*** (82 – 87)	***62*** (76.8 – 83.3)
Reticulocyte (%)	***2.8*** (0.7 – 2.2)	0.8 (0.8 – 2.0)	2.2 (1.1 – 2.9)	1 (0.8 – 2.0)
Iron studies				
Iron (mg/dL)	60 (50 – 120)	62 (40 – 100)	53 (40 – 100)	63 (25 – 150)
TIBC (mg/dL)	293 (250 – 420)	381 (250 – 420)	343 (250 – 420)	313 (240 – 448)
Ferritin (mg/L)	18 (10 – 320)	14 (10 – 375)	38.5 (10 – 375)	18 (20 – 300)
Transferrin (mg/dL)	217 (203 – 360)	272 (203 – 360)	NA	NA
Soluble transferrin receptor[#]	***37.7*** (10.9 – 16.6)	***54.4*** (10.9 – 16.6)	NA	***54.6*** (10.9 – 16.6)

The patients are numbered according to the pedigree in Supplementary Figure 1. Parenthetical values correspond to the age-matched normal ranges from the Clinical Laboratory Improvement Amendments (CLIA)-approved standards for Boston Children's Hospital (for patients A1–A3) and University Hospitals of Cleveland (for patient B1). Values outside the normal range are in bold and italics. MCV, Mean corpuscular volume; TIBC, total iron-binding capacity; NA, not available. [#]Normal range derived from five healthy donors assayed in tandem with the patients.

Supplementary Table 3. Patients' serum immunoglobulin (Ig) levels and lymphocyte profiles.

Patient	**A1**	**A2**	**A3**	**B1**
Age	**13 months**	**5 months**	**2 months**	**5 years**
Immunoglobulin (mg/dL) before IVIG replacement				
IgG	***58*** (294 – 1069)	***77*** (172 – 814)	***191*** (251 – 906)	On IVIG
IgA	***7*** (16 – 84)	***7*** (8.1 – 8.4)	***23*** (1.3 – 53)	***<8*** (25 – 154)
IgM	***27*** (49 – 149)	***26*** (33 – 108)	***16*** (20 – 87)	***<5*** (43 – 196)
Age	**6 years**	**8 months**	**3 months**	**5 years**
Neutrophils (cells/μL)	2770 (2770 – 6340)	***1660*** (2470 – 6410)	***1340*** (2220 – 7110)	3150 (2770 – 6340)
Lymphocytes (cells/μL)	1680 (1230 – 2690)	4980 (2320 – 5490)	***6320*** (1880 – 5390)	2480 (1230 – 2690)
$CD3^+$ (cells/μL)	1386 (1000 – 2600)	3494 (1900 – 6200)	5124 (2500 – 5600)	1643 (1000 – 2600)
$CD3^+CD4^+$ (cells/μL)	815 (530 – 1500)	2502 (1400 – 4300)	3804 (1600 – 4000)	1145 (530 – 1500)
$CD3^+CD8^+$ (cells/μL)	525 (330 – 1100)	841 (500 – 1700)	1147 (500 – 1700)	473 (330 – 1100)
$CD3^-CD16^+/CD56^+$ (cells/μL)	139 (70 – 480)	296 (160 – 1100)	280 (160 – 1100)	274 (70 – 480)
$CD19^+$ (cells/μL)	***152*** (270 – 860)	1550 (610 – 2600)	1183 (430 – 3000)	548 (270 – 860)*
$CD19^+CD27^-IgD^+$ (%)	***94.6*** (47.3 – 77.0)	***97.9*** (85.5 – 93.4)	95.2 (82.1 – 95.2)	***97*** (47.3 – 77.0)*
$CD19^+CD27^+IgD^+$ (%)	***2.1*** (5.2 – 20.4)	***1.7*** (2.8 – 7.4)	3.9 (2.5 – 8.7)	***0.8*** (5.2 – 20.4)*
$CD19^+CD27^+IgD^-$ (%)	***0.2*** (10.9 – 30.4)	***0.1*** (1.6 – 7.0)	***0.2*** (0.3 – 9.0)	***0.4*** (10.9 – 30.4)*

The patients are numbered according to the pedigree in Supplementary Fig. 1. Serum Ig levels shown were measured at the time of disease presentation. Parenthetical values correspond to the age-matched normal ranges from the Clinical Laboratory Improvement Amendments (CLIA)-approved standards for Boston Children's Hospital (for patients A1-A3) and University Hospitals of Cleveland (for patient B1). Bold italicized values are outside the normal range. IVIG, intravenous immunoglobulin; NA, not available. *These laboratory values were obtained before initiation of therapy with anti-CD20 monoclonal antibody.

Supplementary Table 4. Databases and samples queried for the c.58T>C mutation in *TFRC*.

dbSNP v142
61,486 exomes from the Exome Aggregation Consortium (v0.2)
6,503 exomes from the NHLBI Exome Sequencing Project
2,577 genomes from the 1000 Genomes Project (October 2014 release)
662 exomes from the NHGRI ClinSeq project
95 exomes from the NIEHS Environmental Genome Project
69 public genomes sequenced at Complete Genomics, Inc.
In-house collection of 731 unaffected control subjects comprised of 396 ancestry-matched subjects from Kuwait, 81 from the United Arab Emirates, 2 from Saudi Arabia, and 252 of European ancestry

NHLBI, National Heart, Lung, and Blood Institute; NHGRI, National Human Genome Research Institute; NIEHS, National Institute of Environmental Health Sciences.

Supplementary Table 5. Hemogram, lymphocyte subsets, and immunoglobulin levels in *Tfrc*$^{Y20H/Y20H}$ and wild type (WT) mice.

	WT	*Tfrc*$^{Y20H/Y20H}$
Hemogram		
Hemoglobin (g/dL)	14.6 ± 0.25	***12.2*** ± 0.15***
Mean corpuscular volume (fL)	51.2 ± 0.57	***35.6*** ± 1.1***
Platelets (10^3 cells/μL)	916 ± 56	975 ± 34
WBC (10^3 cells/μL)	9,412 ± 720	10,320 ± 1100
Neutrophils (10^3 cells/μL)	1,656 ± 382	1,968 ± 241
Lymphocytes (cells/μL)	6,650 ± 941	7,944 ± 952
$CD3^+$ (% of lymphocytes)	37.4 ± 6.2	33.0 ± 5.5
$CD4^+$ (% of lymphocytes)	17.6 ± 2.8	18.7 ± 3.3
Naïve $CD4^+CD44^{lo}CD62L^{hi}$ (% of $CD4^+$ cells)	42.7 ± 11.9	48.5 ± 4.4
Effector memory $CD4^+CD44^{hi}CD62L^{lo}$ (% of $CD4^+$ cells)	42.7 ± 9.7	37.5 ± 3.3
Central memory $CD4^+CD44^{hi}CD62L^{hi}$ (% of $CD4^+$ cells)	4.2 ± 0.7	4.9 ± 1.4
$CD8^+$	10.5 ± 1.7	9.7 ± 1.2
Naïve $CD8^+CD44^{lo}CD62L^{hi}$ (% of $CD8^+$ cells)	51.1 ± 8.2	55.5 ± 3.2
Effector memory $CD8^+CD44^{hi}CD62L^{lo}$ (% of $CD8^+$ cells)	15.8 ± 4.8	9.0 ± 0.8
Central memory $CD8^+CD44^{hi}CD62L^{hi}$ (% of $CD8^+$ cells)	20.5 ± 6.1	27.6 ± 4.7
Regulatory T cells $CD4^+CD25^+FOXP3^+$	8.03 ± 0.4	7.3 ± 0.5
$B220^+$ (% of lymphocytes)	55.3 ± 5.7	60.1 ± 4.8
$NK1.1^+$ (% of lymphocytes)	2.2 ± 0.2	1.6 ± 0.2
iNKT $TCR\beta^+$α-$GalCer^+$ (% of lymphocytes)	2.2 ± 0.6	1.7 ± 0.3
Immunoglobulin levels (μg/mL)		
IgG	1,033.0 ± 43.7	***356.0*** ± 65.9***
IgA	113.3 ± 14.4	108.4 ± 8.06
IgM	73.9 ± 4.6	64.8 ± 4.8

Mean ± SEM shown for all values. Studies were done on 4–5 mice (4–6 months old) of each genotype. WBC, white blood cells. ***P<0.001. Bold italicized values are outside the normal range.

Supplementary Table 6

Primer Name	Sequence
Sense activation induced cytidine deaminase (*AICDA*)	5’ GAGGCAAGAAGACACTCTGG 3’
Antisense *AICDA*	5’ GTGACATTCCTGGAAGTTGC 3’
Sense Iμ	5’ CTGTTCCGAATCACCGATGC 3’
Antisense Cε	5’ CAGTCTGTGGACGATGGAGT 3’

Supplementary Note

Linkage analysis. Preliminary linkage scans of multiple subsets of Family A, collectively including all individuals, yielded consensus evidence of linkage exclusively to an interval on chromosome 3q28-29. A scan using only five affected subjects and their parents confirmed the linkage to chromosome 3q28-29 at a LOD score of 5.418. To further validate this linkage peak we used SimWalk2 in the full pedigree on a subset of markers spanning the chromosome 3q28-ter interval, and we attained an approximate LOD score of 7.269 in the region.

Positional candidate gene analysis. The chromosome 3q28-29 linkage peak included 19 genes. Copy number analysis revealed no evidence of a copy number mutation within this interval. We Sanger sequenced the protein-coding exons of all 19 genes in an affected individual and found no homozygous nonsynonymous or splice site variants that segregated with the phenotype in all family members and were absent from unaffected control subjects. By qRT-PCR on all 19 genes in whole blood total RNA, we were unable to identify any genes with reproducibly and significantly different expression levels in one patient relative to three controls.

***TFRC*:c.58T>C segregation analysis**. The telomeric portion of chromosome 3 that contains *TFRC* but is distal to the 3q28-29 interval was excluded from the linkage peak solely by subject 32, an obligate heterozygous carrier for the pathogenic mutation. Although he was confirmed unaffected, he had the same homozygous SNP genotypes across the chromosome 3q29-ter region as the affected patients. Accordingly, omission of subject 32's genotypes from the linkage scan resulted in an interval spanning the entire chromosome 3q28-ter region, including *TFRC*.

The *TFRC*:c.58T>C mutation was heterozygous in all obligate carriers, including subject 32, despite the mutation's position within an otherwise homozygous background in that subject. This unexpected observation can be explained if the mutation occurred *de novo* in a common ancestor shared by all obligate carriers in the pedigree, with subsequent inheritance by subject 32, due to consanguinity in the family, of both the mutant haplotype and a non-mutant copy of the otherwise identical haplotype from a more distant ancestor. Though we cannot formally rule out the alternative that subject 32 had a *de novo* reversion of the mutant allele to the reference allele on one of his haplotypes, this possibility is exceedingly unlikely. Regardless, the two nearly identical haplotypes in subject 32 could not be distinguished from each other with the SNP genotype data used in the linkage scans, and therefore, the apparent exclusion of chromosome 3q29-ter from the minimum consensus linkage interval was artifactual. Furthermore, this anomaly of the haplotype inheritance provides strong evidence that the c.58T>C mutation in *TFRC* is pathogenic, since within the chromosome 3q28-ter candidate region the only difference in genotypes between unaffected subject 32 and the affected subjects was that subject 32 was heterozygous and the patients were homozygous for this mutation.