TCF3 haploinsufficiency defined by immune, clinical, gene-dosage, and murine studies

Brigette Boast, PhD[1,*], **Shubham Goel, PhD**[1,*], **Luis I. González-Granado, MD**[2], **Julie Niemela, MS**[1], **Jennifer Stoddard, BS**[1], **Emily S.J. Edwards, PhD**[3], **Sandali Seneviratne, BSc**[4], **Dominik Spensberger, PhD**[5], **Juan F. Quesada, MD**[6], **Luis M. Allende, MD**[7], **John McDonnell, MD**[8], **Alexandria Haseley, MD**[9], **Harry Lesmana, MD**[10], **Magdalena A. Walkiewicz, PhD**[11], **Emad Muhammad, MD**[12], **Julian J. Bosco, MD**[13], **Thomas A. Fleisher, MD**[1], **Shai Cohen, MD**[14], **Steven M. Holland, MD**[11], **Menno C. van Zelm, PhD**[3,13], **Anselm Enders, MD, PhD**[4], **Hye Sun Kuehn, PhD**[1], **Sergio D. Rosenzweig, MD, PhD**[1]

[1]Immunology Service, Department of Laboratory Medicine, National Institutes of Health Clinical Center, Bethesda, USA;

[2]Hospital 12 de Octubre, Madrid, Spain;

[3]Department of Immunology, Monash University, Melbourne, Australia;

[4]Centre for Personalised Immunology and Division of Immunity, Inflammation and Infection, John Curtin School of Medical Research, The Australian National University, Canberra, Australia;

[5]ANU Gene Targeting Facility, Australian Phenomics Facility, John Curtin School of Medical Research, Australian National University, Canberra, Australia;

[6]Genetics Department, Hospital 12 de Octubre, Madrid, Spain;

[7]Immunology Department, Hospital 12 de Octubre, Madrid, Spain;

[8]Department of Pediatric Allergy and Immunology, Cleveland Clinic, Cleveland, USA;

[9]Center for Personalized Genetic Healthcare, Genomic Medicine Institute, Cleveland Clinic, Cleveland, USA;

[10]Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation, Cleveland Clinic, Cleveland, USA;

Corresponding Author: Sergio D. Rosenzweig, Immunology Service, Department of Laboratory Medicine, National Institutes of Health Clinical Center, sergio.rosenzweig@nih.gov, (301) 770-0797.

*B.B. and S.G. contributed equally to this study.

[11]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA;

[12]Hematology Laboratory, Carmel Medical Center, Haifa, Israel;

[13]Allergy, Asthma and Clinical Immunology Service, Alfred Hospital, Melbourne, VIC, Australia;

[14]Allergy and Clinical Immunology Service, Department of Internal Medicine B, Lin & Carmel Medical Center, The Technion, Israel Institute of Technology, Haifa, Israel

Abstract

Background: TCF3 is a transcription factor contributing to early lymphocyte differentiation. Germline monoallelic dominant negative (DN) and biallelic loss-of-function (LOF) Null *TCF3* mutations cause a fully penetrant severe immunodeficiency. We identified 8 individuals from 7 unrelated families with monoallelic LOF *TCF3* variants presenting with immunodeficiency with incomplete clinical penetrance.

Objective: To define TCF3 haploinsufficiency biology and its association with immunodeficiency.

Methods: Patient clinical data and blood samples were analyzed. Flow cytometry, western blotting, plasmablast differentiation, immunoglobulin secretion, and transcriptional activity studies were conducted on individuals carrying *TCF3* variants. Mice with a heterozygous *Tcf3* deletion were analyzed for lymphocyte development and phenotyping.

Results: Individuals carrying monoallelic LOF *TCF3* variants showed B cell defects (e.g., reduced total, class-switched memory and/or plasmablasts), and reduced serum immunoglobulin levels; most but not all presented with recurrent, non-severe infections. These *TCF3* LOF variants were either not transcribed or translated, resulting in reduced wildtype TCF3 protein expression, strongly suggesting haploinsufficiency (HI) pathophysiology for the disease. Targeted RNAseq analysis of T cell blasts from *TCF3* Null, DN, or HI individuals clustered away from healthy donors, implying that two WT copies of *TCF3* are needed to sustain a tightly regulated *TCF3* gene-dosage effect. Murine TCF3 HI resulted in a reduction of circulating B cells but overall normal humoral immune responses.

Conclusion: Monoallelic LOF *TCF3* mutations cause a gene-dosage-dependent reduction in wildtype protein expression, B cell defects and a dysregulated transcriptome, resulting in immunodeficiency. $Tcf3^{+-/-}$ mice partially recapitulate the human phenotype underscoring differences between TCF3 in humans and mice.

Capsule summary

Germline monoallelic loss-of-function variants manifest as a predominantly antibody deficiency with incomplete clinical penetrance and present a milder form of disease when compared to biallelic LOF and monoallelic DN *TCF3* variants. The definition of TCF3 haploinsufficiency adds to the previously known body of literature describing *TCF3* variants in PID/IEI and B-ALL cohorts.

Keywords

primary immunodeficiency; inborn errors of immunity; E47; E12; E2A; predominantly antibody deficiency; common variable immunodeficiency; gene-dosage; hypogammaglobulinemia; haploinsufficiency

Introduction

E proteins are members of the basic helix loop helix (bHLH) family of transcription factors that have essential roles in lymphocyte development.[1] They bind to E box CANNTG DNA sequences as heterodimers or homodimers, exerting different transcriptional profiles that establish alternate lymphoid lineages. There are four members of the E protein family: E12, E47, E2–2 (TCF4), and HEB (TCF12). E12 and E47 are generated by alternative splicing of the C' terminal region of the same gene, *E2A* (*TCF3*), and only differ by one exon located within the bHLH region.[2] Whilst there is no difference in the DNA binding specificity between E12 and E47, E12 includes a small inhibitory region that interferes with homodimerization and DNA binding efficiency, but does not interfere with heterodimerization with E47 or other bHLH proteins.[2] E47 on the other hand, efficiently binds to DNA as a homodimer, in a B lineage-restricted manner.[3] Studies in mice show some redundant and some unique roles for E12 and E47 in B cell development.[4]

In mice, TCF3 is first expressed in developing lymphocytes in the bone marrow, with the earliest expression detected in lymphoid primed progenitors (LMPPs). Here, TCF3 aids in establishing differentiation to common lymphoid progenitors (CLPs) by upregulating lymphoid-specific genes.[5] TCF3-deficient mice have no lymphocytes as a consequence of being unable to correctly express the lymphoid gene signature in LMPPs.[5–7] Murine studies have shown that during B and T cell development, TCF3 also has essential roles in establishing both B and T cell identity in the bone marrow and thymus respectively. In the bone marrow, TCF3 expression remains high throughout precursor B cell development. During pro-B cell development, TCF3 activates EBF1 to initiate the B cell-specific transcriptional profile, including the up-regulation of PAX5 by EBF1.[8–10] Additionally, TCF3 directly modulates RAG expression during immunoglobulin heavy and light chain gene rearrangement[11] allowing for progression through both pro- and pre-B cell checkpoints.[10] Once BCR check-point signaling has been cleared in the bone marrow, IgM$^+$ cells will migrate to the spleen where they downregulate TCF3 expression[10] and complete maturation through the transitional B cell stages (T1-T3). Here, it appears TCF3 expression is not essential in mice, despite it having some effects on mature follicular B cell (FoB) survival.[10] In addition, reduced expression of TCF3 appears to promote marginal zone B cell (MZB) development.[12] Upon T-dependent activation, TCF3-deficient B cells are able to initiate germinal centers (GC) but at significantly reduced numbers.[10, 13, 14] Although E proteins have been shown to be essential for class-switch recombination by initiating *Aicda* expression,[15] this process appears to be redundant in mice as the ability to isotype switch and produce high-affinity antibodies is maintained when TCF3 is conditionally deleted in mature B cells.[10]

Previously, monoallelic dominant negative (DN) and biallelic loss-of-function (LOF, Null) *TCF3* variants have been associated with fully penetrant syndromic and combined immunodeficiency forms of disease, respectively.[16–20] A monoallelic DN variant, E555K, that only affects E47, causes an incomplete arrest of B cell development in the bone marrow prior to the pro-B cell stage and agammaglubulinemia.[16, 17, 20] The few B cells that are present in the periphery of these patients lack a BCR and have increased expression of CD19.[16, 17] Biallelic LOF variants (c.808 C>T, p.Q270* and an out-of-frame exon 5–11 deletion) resulting in Null *TCF3* expression also cause immune defects with the clinical phenotype consisting of severely reduced B cells and agammaglobulinemia, recurrent bacterial infections, facial dysmorphia characterized by overfolded ear helixes and prominent antihelix stems, as well as an increased risk for developing pediatric B-ALL.[18, 19]

Here, we present a cohort of unrelated individuals with heterozygous LOF *TCF3* variants defining TCF3 haploinsufficiency at a genetic, transcriptional, protein expression, and clinical level.

Methods

Patients suspected of having a PID/IEI and their relatives were evaluated for this study (Families B, C, F, G and H). Patients known to carry disease-causing TCF3 mutations, and their relatives were also tested (Families A, D and E). All research subjects or their guardians provided written informed consent in accordance with the Declaration of Helsinki under institutional review board–approved protocols at their primary care centers, as the Alfred Health 109/15 and Monash University CF15/771-2015-0344 protocols, or the NIH/NIAID protocol 10-I-0216; ClinicalTrials.gov Identifier: NCT01222741. Peripheral blood samples from patients and their relatives were immunophenotyped and functionally evaluated. Genomic DNA was extracted, tested by whole exome sequencing, and prioritized variants were Sanger confirmed; cDNA was also sequenced, either directly after mRNA/cDNA conversion or after subcloning. PBMCs were collected and used for protein immunoblotting, as well as in-vitro plasmablast differentiation and immunoglobulin secretion. Transcriptomic activity was evaluated by RNASeq on CD4+ T cell blasts; plasmids containing different TCF3 variants were generated, and transcriptional luciferase activity was tested after transfection of HEK293T cells. C57BL6/N *Tcf3*$^{+/-}$ and *Tcf3*$^{+/S513*}$ mice were generated by CRISPR/Cas9 methodology, immunophenotyped and functionally evaluated. More detailed materials & methods can be found on the supplementary appendix.

RNAseq data may be found in the GEO repository, accession ID: GSE218787. *Tcf3*$^{+/-}$ and *Tcf3*$^{+/S513*}$ mice are available through the Australian Phenome Bank (https://pb.apf.edu.au).

Results

Clinical, immunological, and genetic characterization

Eight different *TCF3* variants, including the previously reported DN E555K (Family D), were identified in thirteen individuals from eight unrelated families (Figure 1A,B). While the variants detected in Families A-G affected single nucleotides or small indels at both the N' and C' gene termini, the index in Family H had a large, heterozygous

chromosomal deletion (Ch37/hg19 19p13.3(chr19: 959646–1649320)x1) that spanned 38 genes including exons ~3–21 of *TCF3* (information on the other affected genes can be found in Supplementary Table 1). In-depth clinical summaries for each patient can be found in the supplemental data. Previously, only patients with biallelic LOF/Null and monoallelic DN (E555K) variants of *TCF3* have been described.[16–20] Here, we observed monoallelic LOF *TCF3* mutations (n=8) associated with a milder form of a predominantly antibody deficiency presenting in children or adults with reduced total B cells, switched-memory B cells and/or plasmablasts, hypogammaglobulinemia and increased susceptibility to infections with incomplete penetrance (asymptomatic = 3/8) (Figure 1C,D; Table 1) In contrast, Null (n=4) and DN (n=1) mutations caused a more severe and broader phenotype with facial dysmorphia (Null, Supplemental Figure 1), recurrent severe infections, susceptibility to B-ALL, and reduced circulating B cells, class-switched memory B cells, and plasmablasts (Table 1). Additionally, individuals with HI mutations had normal Igκ usage whilst those with Null mutations had a skewed Igκ:Igλ ratio (Figure 1E); of note, the patient with the DN mutation did not express a BCR.

TCF3/E2A protein expression and gene transcription

Protein expression of TCF3 was reduced in all TCF3-mutated individuals, except for the patient with the previously described DN E555K variant (D.II.1), who had normal TCF3 expression (Figure 2A,B). Null mutations in *TCF3* completely abolished TCF3 protein expression, whilst monoallelic LOF mutations showed intermediate levels of protein (Figure 2B), strongly suggesting HI of TCF3 as the mode of disease pathophysiology in these individuals. *TCF3* mRNA was evaluated either by bulk cDNA or subcloning analysis from T cell blasts derived from all described variants (except for H.II.1). No mutant transcripts were detected in Family G (Supplemental Figure 2), strongly suggesting that the observed reduced protein expression was the result of mutant *TCF3* mRNA decay. Although protein accumulation was markedly decreased, mutant cDNA transcripts were detected in members of Families A, B, C, E, and F (Supplemental Figure 2) suggesting that reduced stability of the mutant protein is the likely mechanism underlying this finding. As expected, mutant and WT transcripts were detected in Family D index patient (DN mutation), supporting previously reported results of stably expressed mutant transcript and protein.[17] Lastly, although the mutation site identified in Families A and G was the same (c.1451–18A), the different nucleotide substitutions generated a stable mutant transcript in Family A and no mutant transcript in Family G, yet both variants resulted in reduced TCF3 protein expression.

Functional defects of TCF3 transcript-positive mutations

Although mutant proteins were not detected in any of the 5 Families showing mutant transcripts (Families A, B, C, E and F), we still explored the potential transcriptional role of three of these predicted proteins: the intronic splice mutation leading to p.G486Lfs*4 in Family A, the exonic mutation leading to p.S514* in Family B, and the one nucleotide deletion leading to D580Afs*29 in family F and compared these to the known DN variant, E555K. Mutant constructs coding for predicted proteins p.G486Lfs*4 (Family A), p.S514* (Family B), and p.D580Afs*29 as well as the efficiently-detected DN variant E555K (Family D), were cloned into N-terminal-Flag tagged E47 vectors and tested in a

luciferase assay to evaluate the regulation of the TCF3-target gene *MCK*, in single mutant or WT/mutant co-transfection experiments (Supplemental Figure 3). While all mutants were efficiently overexpressed in the transfection system, they lost their ability to upregulate *MCK* as compared to the WT vector in the single transfection experiments. When the mutant vector coding for E555K was co-transfected with the WT vector, it exerted a DN effect over the WT protein activity that was not observed when Family A, B, or F variants were co-transfected with the WT allele. Therefore, any hypothetical residual TCF3 protein expression from mutant alleles in Families A, B, or F would result in LOF with no evidence of a DN effect, further supporting HI as a mechanism of disease for these variants (Supplemental Figure 3). Whilst we were unable to clone cDNA constructs from Family E and were unable to detect mutant transcript from Families G and H, the absence of mutant protein detected in these individuals suggest that these variants create unstable transcripts also supporting an HI mechanism of disease.

Plasmablast differentiation and immunoglobulin production in TCF3-mutated individuals

Unlike Null or DN mutations, HI mutations in *TCF3* do not appear to cause significant reductions in peripheral B cell numbers in most subjects. Individuals with TCF3 HI do, however, tend to have a reduction in total B cells (CD19$^+$), switched-memory B cells (CD20$^+$CD27$^+$IgM$^-$) and plasmablasts (CD19$^+$CD24$^-$CD38^{++}) as also described in Null and DN individuals (Figure 1D, Table 1). B and T cell activation and proliferation in response to in vitro CpG and IgM, CD40L and IL-21, or αCD3/αCD28 stimulation were normal in all *TCF3*-mutated individuals (Table 1).

In vitro plasmablast differentiation, however, was impacted in almost all mutated individuals tested. Patients with Null or the monoallelic DN variants, produced virtually no plasmablasts in response to CD40L and IL-21 stimulation (Figure 3A). However, individuals with HI variants were able to produce approximately 50% of the number of plasmablasts compared to healthy controls (Figure 3A). Similarly, supernatant collected from in vitro stimulation with CD40L and IL-21 showed that biallelic *TCF3* Null or DN mutations lead to virtually absent immunoglobulin production. In contrast, the heterozygous HI mutations lead to an approximate 50% reduction in immunoglobulin levels when compared to healthy controls (Figure 3B). No statistically significant differences were observed when asymptomatic and symptomatic HI individuals were treated as separate groups (data not shown) suggesting no immunological prediction as to whether two individuals carrying the same heterozygous HI variant will develop clinical symptoms. Taken together, HI variants in *TCF3* halve protein expression and are associated with decreased plasmablast differentiation and immunoglobulin production compared to healthy controls, demonstrating a *TCF3* gene dosage effect.

Transcriptomic analysis of TCF3-deficient T cells

To understand how mutations in *TCF3* confer immune dysregulation in a gene dosage-dependent manner, we compared the transcriptomic landscape from individuals carrying Null, DN, and heterozygous HI *TCF3* mutations (Figure 4). As B cells were markedly reduced or virtually absent in patients with Null and DN *TCF3* mutations, we studied activated CD4$^+$ T cells, a cell lineage present and responsive to activation in all *TCF3*

allelic variants. When filtered down to genes that were differentially expressed across the *TCF3*-mutated individuals tested, all *TCF3* mutation positive individuals clustered together regardless of their underlying genetic variant and separated from the healthy controls, suggesting that two WT *TCF3* alleles are required for normal gene transcription (Figure 4A). Pathway analysis of differentially expressed genes showed that Null and DN individuals overexpressed genes involved in the recruitment of leukocytes, activation of cells, hypersensitivity reactions, and proliferation of immune cells, whereas the HI individuals underexpressed genes involved in those functions. In contrast, all three allelic variants similarly overexpressed genes involved in accumulation of leukocytes, invasion of cells, leukocyte migration, chemotaxis, homing of cells, hematologic cancer, lymphocyte homeostasis, and T cell development (Figure 4B). This shows that *TCF3* allelic variants can regulate specific pathways in similar or different directions and, as shown in T-cell blasts, two *TCF3* wildtype copies are needed to sustain normal transcriptomic activity. Moreover, transcriptomic abnormalities did not cosegregate with clinical penetrance as asymptomatic individuals (A.I.1 and A.I.2) demonstrated no unique transcriptomic patterns when compared with symptomatic HI individuals (B.II.1 and C.I.1) or Null (A.II.2) and DN (D.II.1) individuals (Figure 4A).

Haploinsufficiency of TCF3 in mice

To further explore the gene dose-dependent effects of *TCF3* mutations on B cell function, we used CRISPR/Cas9 to delete *Tcf3* in the germline of C57 BL/6 mice. A complete absence of *Tcf3* in the germline is incompatible with life, with homozygous pups dying soon after birth[6, 7, 21] and as such, mice were only bred to heterozygosity ($Tcf3^{+/-}$). Haploinsufficiency of TCF3 caused a significant reduction in the frequency of circulating B cells in the blood (Figure 5A). Total cell numbers in the bone marrow (BM) were unaffected, but there was a significant reduction of B cells, including precursor, immature, and mature B cells due to a partial blockage of cells progressing to the pro-B cell stage (Figure 5B). B cell frequencies in the spleen were reduced in $Tcf3^{+/-}$ mice, including a reduction in follicular B cells (FoB) and in immature B cells migrating from the bone marrow (Figure 5C). B cell maturation through transitional B cell stages was however, unaffected, suggesting normal B cell maturation. Switched (IgM^-IgD^-) B cells were normal in $Tcf3^{+/-}$ mice but marginal zone (MZ) B cells were slightly increased. The proportion of B cells expressing $Ig\lambda$ was very significantly reduced in $Tcf3^{+/-}$ B cells (Figure 5C) confirming previous reports that TCF3 affects $Ig\lambda$ locus accessibility.[4, 12] Total and $CD4^+$ T cells in the spleen were unaffected in $Tcf3^{+/-}$ mice. These results confirm that murine TCF3 haploinsufficiency results in a significant impact on B cell biology stemming from an inability for B cells to correctly progress to the pro-B cell stage within the BM.[5, 12, 22]

Additionally, we used CRISPR/Cas9 to mimic the S514* mutation found in Family B and created mice heterozygous for the homologous S513* mutation ($Tcf3^{+/S513*}$). This showed a similar reduction in circulating B cells and a reduced frequency of $Ig\lambda^+$ B cells (Supplemental Figure 4) confirming the deleterious nature of a monoallelic *Tcf3* nonsense mutation.

To determine the impact of TCF3 HI on the humoral immune response, the ability to form germinal centers after immunization with sheep red blood cells (SRBCs) was analyzed (Figure 5D). Surprisingly, the frequency of GC B cells was not affected in $Tcf3^{+/-}$ mice, yet total numbers were significantly reduced due to lower numbers of total B cells. Both the frequency and number of switched (IgM⁻IgD⁻) B cells were normal in $Tcf3^{+/-}$ mice. Moreover, T follicular helper cells (Tfh) were significantly reduced in both total number and frequency, despite comparable total T cell numbers.

Next, we tested the ability for $Tcf3^{+/-}$ B cells to differentiate into plasma cells in vitro by crossing $Tcf3^{+/-}$ with $Blimp1^{Gfp}$ reporter mice.[23] This showed normal plasma cell frequencies in $Tcf3^{+/-}$ $Blimp1^{Gfp}$ mice as well as normal isotype switching, as frequencies of plasma cells (CD138⁺GFP⁺) and IgG1⁺IgD⁻ B cells were comparable to wildtype following 4 days of in vitro stimulation with LPS ± IL-4 and IL-5 (Figure 5E).

Finally, to test antibody production in the context of murine TCF3 HI, we measured immunoglobulin concentrations in the plasma of naïve mice (Figure 5F). Surprisingly, IgA, IgG2a, IgG3, and IgM were significantly increased in $Tcf3^{+/-}$ mice compared with $Tcf3^{+/+}$ mice, potentially implying a slightly increased activation state in naïve conditions. When challenged with foreign immunogens, immunized $Tcf3^{+/-}$ mice produced antigen-specific antibodies comparable to $Tcf3^{+/+}$ mice (Figure 5G). Immunization with T-dependent chicken gamma globulin (CGG) and *B. pertussis* showed normal CGG-specific IgG1 and slightly increased *B. pertussis*-specific IgG2c in $Tcf3^{+/-}$ mice two weeks post immunization. Immunization with T-independent NP-Ficoll showed normal NP-specific IgM and switched NP-specific IgG3 6 days post immunization in $Tcf3^{+/-}$ mice. Together, this data demonstrates that murine TCF3 HI has significant effects on B cell development stemming from the bone marrow and alters B cell subsets in the periphery, but this loss of mature B cells in the periphery does not impede the humoral immune response.

Discussion

TCF3 deficiency was originally described as an autosomal dominant PID/IEI disease caused by a monoallelic DN mutation (E555K) leading to a virtual absence of B cells and agammaglobulinemia.[16, 17, 20] Later, an autosomal recessive variant of the disease was described, where biallelic LOF mutations leading to Null protein expression were linked to a similar immune phenotype, but also included mild facial dysmorphisms and increased B-ALL susceptibility.[18, 19] Here, we establish TCF3 HI as a new trait for immune deficiency and compare this with previously described *TCF3* allelic variants. By studying *TCF3*-mutated patients and their relatives we determined that TCF3 HI appears to have complete immunologic but incomplete clinical penetrance, as observed in several other PID/IEI based on HI.[24–26] While the reasons underlying the incomplete clinical penetrance for such diseases are not yet fully understood, factors intrinsic to the affected gene (e.g., related to particular cell lineages and thresholds for function), as well extrinsic to the gene, may likely be involved. Whilst all HI individuals described in this report had reduced WT TCF3 and no mutant protein expression, the mechanisms underlying this finding could result from either partial gene deletion, mutant mRNA decay and/or mutant protein instability. Symptomatic TCF3 HI causes a milder immunologic and clinical phenotype

than the other allelic variants, with symptomatic individuals presenting as children or adults with hypogammaglobulinemia, reduced total B cells, switched-memory B cells and/or plasmablasts, as well as recurrent, although not opportunistic or life threatening, infections. Of note, while no malignancies were reported in our TCF3 HI cohort, germline heterozygous LOF variants were detected in 12/4,183 (0.29%) pediatric B-ALL patients.[27] This finding not only suggests that TCF3 HI can include hematologic malignances as part of its phenotype, but also reinforces TCF3 HI as a symptomatic allelic variant and should be considered when determining risk of B-ALL during follow-up evaluation.

Studying individuals with *TCF3* HI together with those carrying DN or Null genotypes and phenotypes allowed us to delineate the role of TCF3 in human B cell biology including a gene-dosage perspective. Besides the reduction of total B cells, switched-memory B cells and plasmablasts associated with all three allelic variants (although not in all TCF3 LOF monoallelic mutation carriers), TCF3 HI causes an intermediate reduction in in vitro plasmablast generation and serum immunoglobulin secretion without affecting B cell activation or proliferation when compared to healthy controls (Figure 1C–E, Table 1). In addition to its known role in B cell development in humans, TCF3 is also upregulated during plasma cell differentiation.[28] It is possible that reduced TCF3 affects the expression of the transcription factor network required for induction of the plasma cell program. Moreover, other TCF3-extrinsic factors such as epigenetic methylation during terminal differentiation of human B cells into plasma cells, also play a role in this function and may even affect penetrance of the disease.[29]

Whilst the immune phenotype of the individual with the chr19 microdeletion that involves 38 genes including *TCF3* exons ~3–21 fits the immune parameters described in the other TCF3 HI individuals, it also includes additional clinical features (e.g., developmental delay, Crohn's-like disease, and Peutz-Jeghers Syndrome) that may depend on other genes compromised in this contiguous gene disorder. In this setting, we can neither absolutely nor formally exclude the potential contributions of some other genes in this micro-deleted region to the immune phenotype of this patient (Supplementary Table 2). Altogether, *TCF3* variants resulting in no mutant protein expression/reduced WT protein expression either by partial gene deletion, mRNA decay, or protein instability strongly support TCF3 HI as the mechanism of disease in the reported individuals.

Heterozygous LOF mutations resulting in *TCF3* HI appear to affect immunoglobulin production in humans more than in mice, as $Tcf3^{+/-}$ mice had normal frequencies of GCBs, in vitro plasmablast differentiation and immunoglobulin, and specific response to antigen stimulation (Figure 5D–G). Whilst B cell development does not appear to be consistently affected by HI mutations in humans, a mild but significant reduction in B cells was observed both in our $Tcf3^{+/-}$ mice and those previously described by other groups.[10, 12, 22, 30, 31] Additionally, the ability to mount a normal humoral response was observed in the murine model (Figure 5D–G) despite a lack of normal immunoglobulin production in the human HI cohort.

Secondary $Ig\lambda$ rearrangements are sensitive to TCF3 dosage in mice,[12] but this does not appear to be the case in humans as those with *TCF3* HI had normal usage of the Igλ light

chain. Individuals with DN or null variants however, had skewed κ:λ ratios corroborating murine studies that TCF3 is essential for chromatin accessibility at the *Igλ* locus.[4, 12]

The lethality observed in *Tcf3*^{−/−} neonatal mice[6, 7, 21] is also not reflected in human cohorts as evidenced by TCF3 Null patients in ours and others' previous studies.[18, 19] The loss of TCF3 therefore has markedly dissimilar impacts on neonatal survival in mice compared with humans, reinforcing TCF3 human/murine differences with human B cells being more sensitive to TCF3 dosage than murine B cells.

Interestingly, two recent papers explored heterozygous *TCF3* mutations that caused B cell defects and reduced antibody production.[32, 33] In one publication, two family members carrying a heterozygous variant in *TCF3* were diagnosed with CVID.[32] Whilst one of the family members also carried a heterozygous C104R variant in *TACI*, the other only carried the *TCF3* T168fs*191 variant. This mutation resulted in nonsense mediated decay with reduced WT protein expression, suggesting HI of TCF3. TCF3 T168fs*191 was also associated with lower IgG production in vitro, normal B cell proliferation and a normal ability for cells to undergo isotype switching. In another report, a patient with a history of recurrent infections, low IgG, slightly increased B cells, and normal numbers of T cells, as well as progressive multifocal leukoencephalopathy, was diagnosed with combined immune deficiency and was reported to carry a heterozygous *TCF3* variant (c.807 C>G, p.H269Q). In this case, TCF3 HI was suspected to be the underlying cause of the disease, although no functional tests were conducted.[33]

In summary, TCF3 HI is an autosomal dominant genetic trait presenting with complete immunologic but incomplete clinical penetrance that underlies a predominantly antibody deficiency.[34] Exploring TCF3 HI in a murine model allowed us to corroborate its impact on B cell development and function, although with substantial differences compared to the human disease. TCF3 HI will likely contribute to elucidate underlying genetic diagnoses and segregation in PID/IEI patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations

B-ALL	B cell acute lymphoblastic leukemia
BCR	B cell receptor
bHLH	basic helix loop helix
BM	bone marrow
CGG	chicken gamma globulin
CLP	common lymphoid progenitor
CVID	common variable immunodeficiency
DN	dominant negative
ELISA	enzyme linked immunosorbent assay
FACS	fluorescence activated cell sorting
FBS	fetal bovine serum
FCS	fetal calf serum
FoB	follicular B cell
GC	germinal center
HI	haploinsufficiency
IEI	inborn errors of immunity
LMPP	lymphoid primed progenitor
LN	lymph nodes
LOF	loss-offunction
LPS	lipopolysaccharide
MZB	marginal zone B cell

NK	natural killer cell
PBMC	peripheral blood mononuclear cells
PID	primary immune deficiency
PSG	penicillinstreptomycin-glutamine
SRBC	sheep red blood cells
T1	transitional 1
T2	transitional 2
T3	transitional 3
Tfh	T follicular helper cells
WES	whole exome sequencing
WT	wildtype

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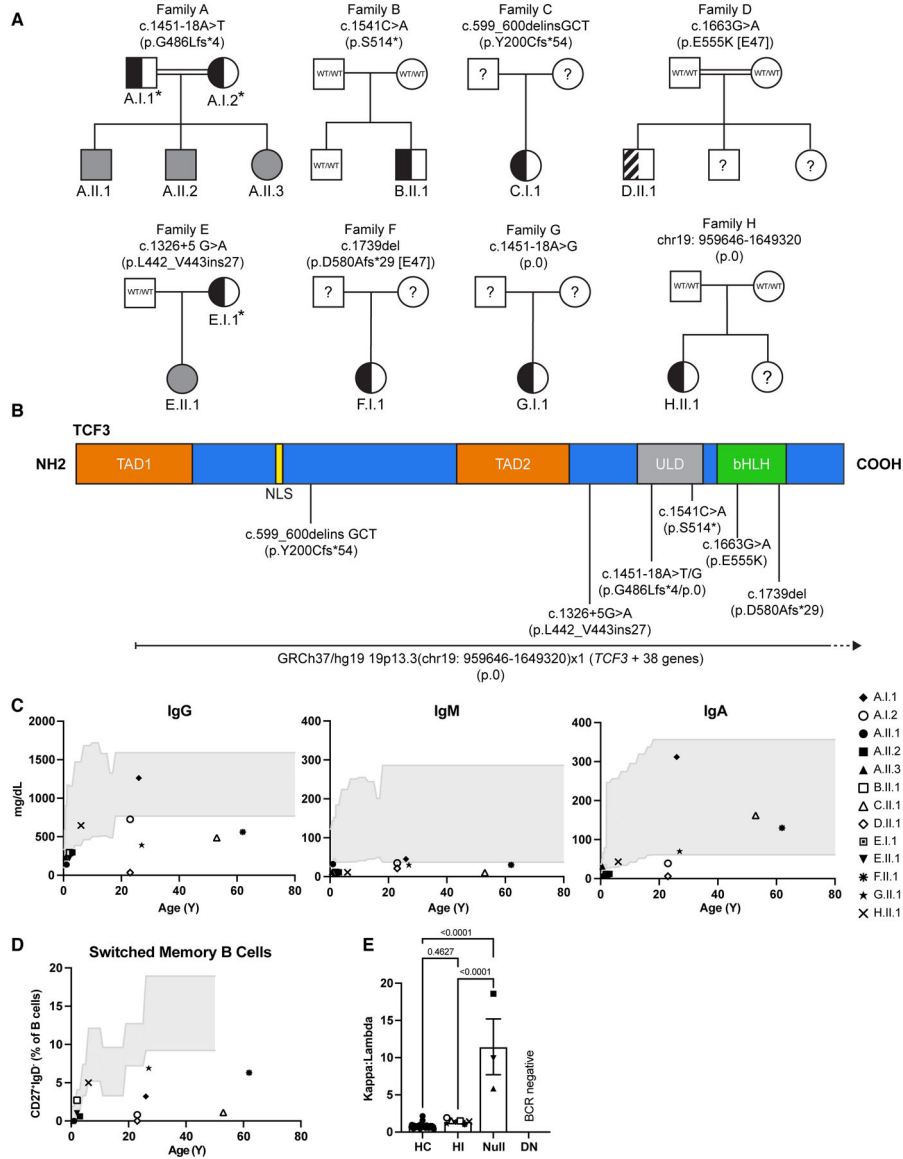
Clinical Implications

Germline monoallelic loss-of-function variants in *TCF3* should be considered as a genetic diagnosis underlying predominantly antibody deficiency in PID/IEI patients.

Figure 1:

Pedigree analysis and clinical manifestations of families with *TCF3* variants. (A) Pedigrees of 8 kindreds with different *TCF3* variants (families A–H). Individuals with heterozygous and homozygous variants are shown in semi-shaded (black) and shaded (gray) symbols respectively, question marks indicate unscreened individual, asterisks indicate mutation positive asymptomatic individuals, the striped semi-shaded square refers to the known DN variant. (B) Schematic representation of the TCF3 protein and corresponding domains. Predicted effect of each mutation on the amino acid sequence and its location are shown. Numbers indicate cDNA and amino acid location. TAD1: transactivation domain 1; NLS: nuclear localization sequence; TAD2: transactivation domain 2; ULD: ubiquitin-like domain; bHLH: basic helix-loop-helix. (C) Immunoglobulin levels in the serum for each tested individual as denoted by unique symbols, compared with healthy control ranges over age (gray shaded area). (D) Frequency of switched memory B cells (CD27$^+$IgD$^-$) in the blood,
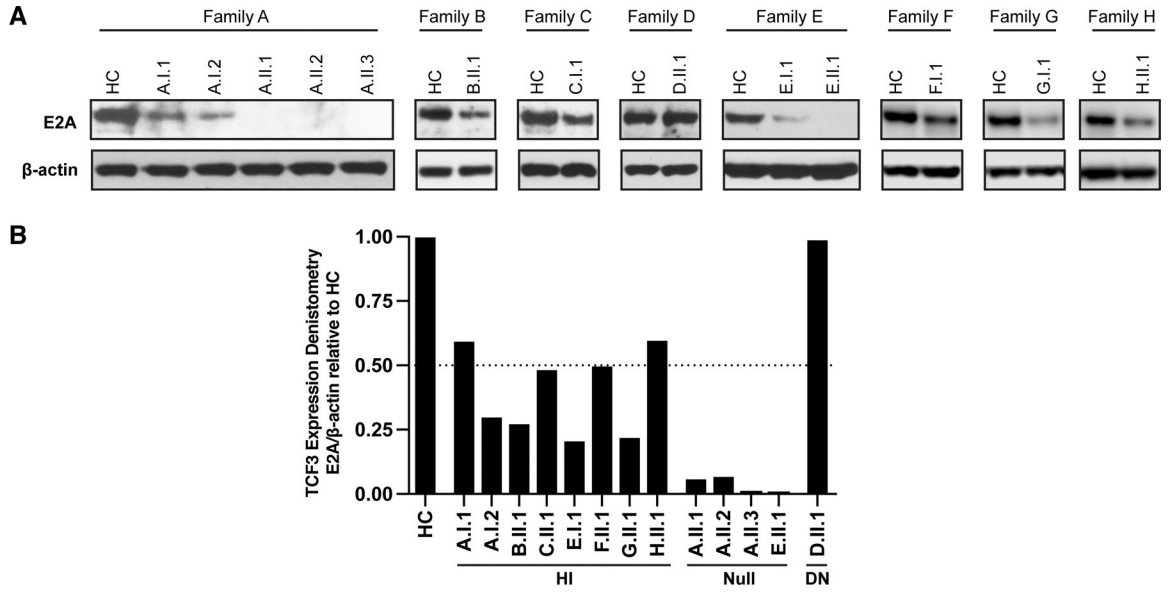
determined by flow cytometry. (E) Igκ to Igλ ratio of B cells in the blood, determined by flow cytometry. Bars show the average for each group ± SEM, one-way ANOVA with Tukey correction, each patient is represented by a unique symbol. HC: healthy control, HI: haploinsufficient, DN: dominant negative.

Figure 2:
TCF3/E2A protein expression in T cell blasts. (A) TCF3/E2A protein expression from T cells blasts from healthy controls (HC) and *TCF3*-mutated individuals. PBMCs were stimulated with anti-CD3/anti-CD28 (1μg/ml each) plus IL-2 (20ng/ml) for 7–12 days followed by protein lysate preparation and immunoblotting with a TCF3/E2A antibody that recognizes both E12 and E47 isoforms. Data shown is representative of two independent experiments. (B) Densitometry of E2A protein expression normalized to β-actin and relative to the HC samples run on the same gel, is shown for TCF3 haploinsufficient (HI), null, and dominant negative (DN) individuals.
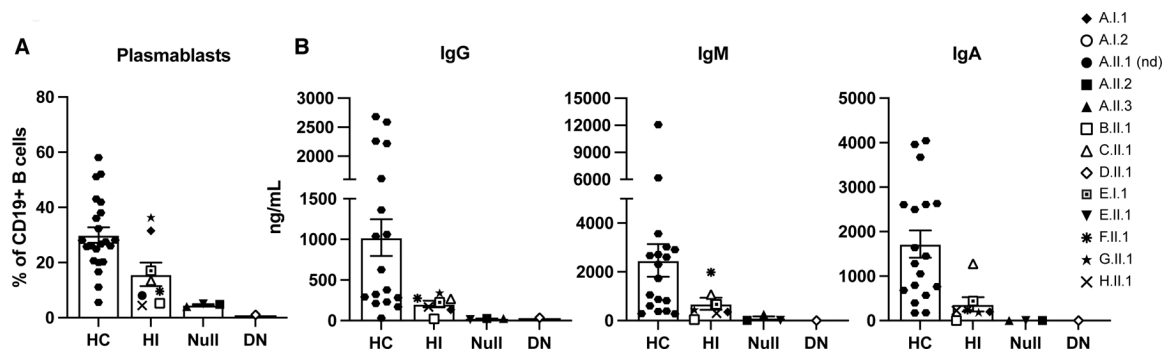
Figure 3:

TCF3 mutations affect in vitro plasmablast differentiation and immunoglobulin production. (A and B) PBMCs were stimulated with CD40L (100 ng/ml) and IL-21(100 ng/ml) for 5 days. (A) Plasmablast frequency in healthy controls (HC), haploinsufficient (HI, monoallelic LOF), Null (biallelic LOF), and dominant negative (DN, monoallelic DN) individuals was determined by the percentage of CD27+CD38+ cells from total CD19+ B cells. (B) Concentration (ng/ml) of immunoglobulin production in the cell culture supernatant post CD40L and IL-21 stimulation determined by ELISA. (A and B) Bars show mean ± SEM of the average of two independent experiments with individuals represented by unique symbols as indicated.
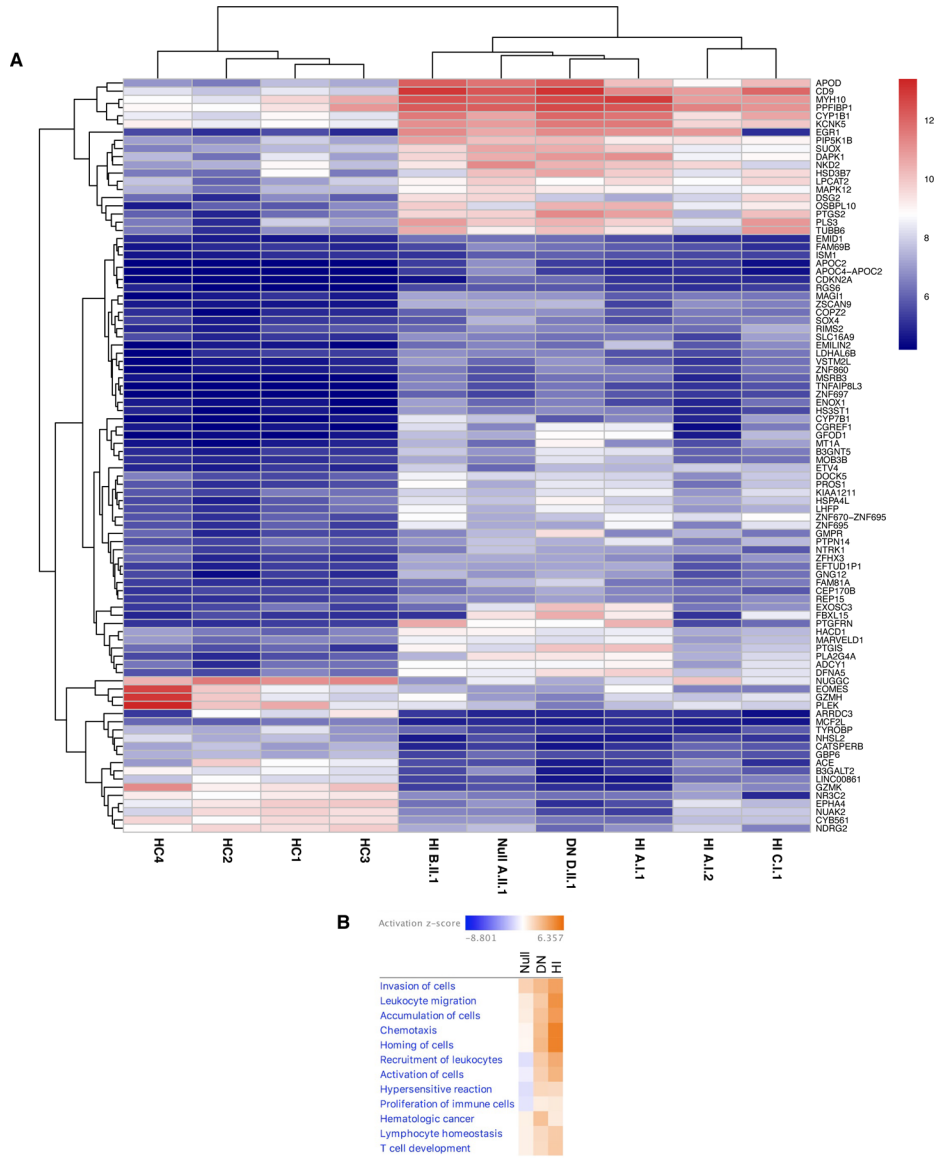
Figure 4:

Null, DN, and HI *TCF3* variants change the mRNA landscape of T cell blasts compared with healthy controls. (A) Heatmap of commonly differentially expressed genes in healthy controls (HC), haploinsufficient (HI), null, and dominant negative (DN) individuals. (B) Ingenuity Pathway Analysis comparing diseases & biofunctions in null, DN, and HI *TCF3* individuals. (A-B) Healthy controls (n=4), TCF3 null (n=1; A.II.1), TCF3 HI (n=4; A.I.1, A.I.2, B.II.1 and C.II.2), TCF3 DN (n=1; D.II.1). Two technical replicates were performed for each individual.
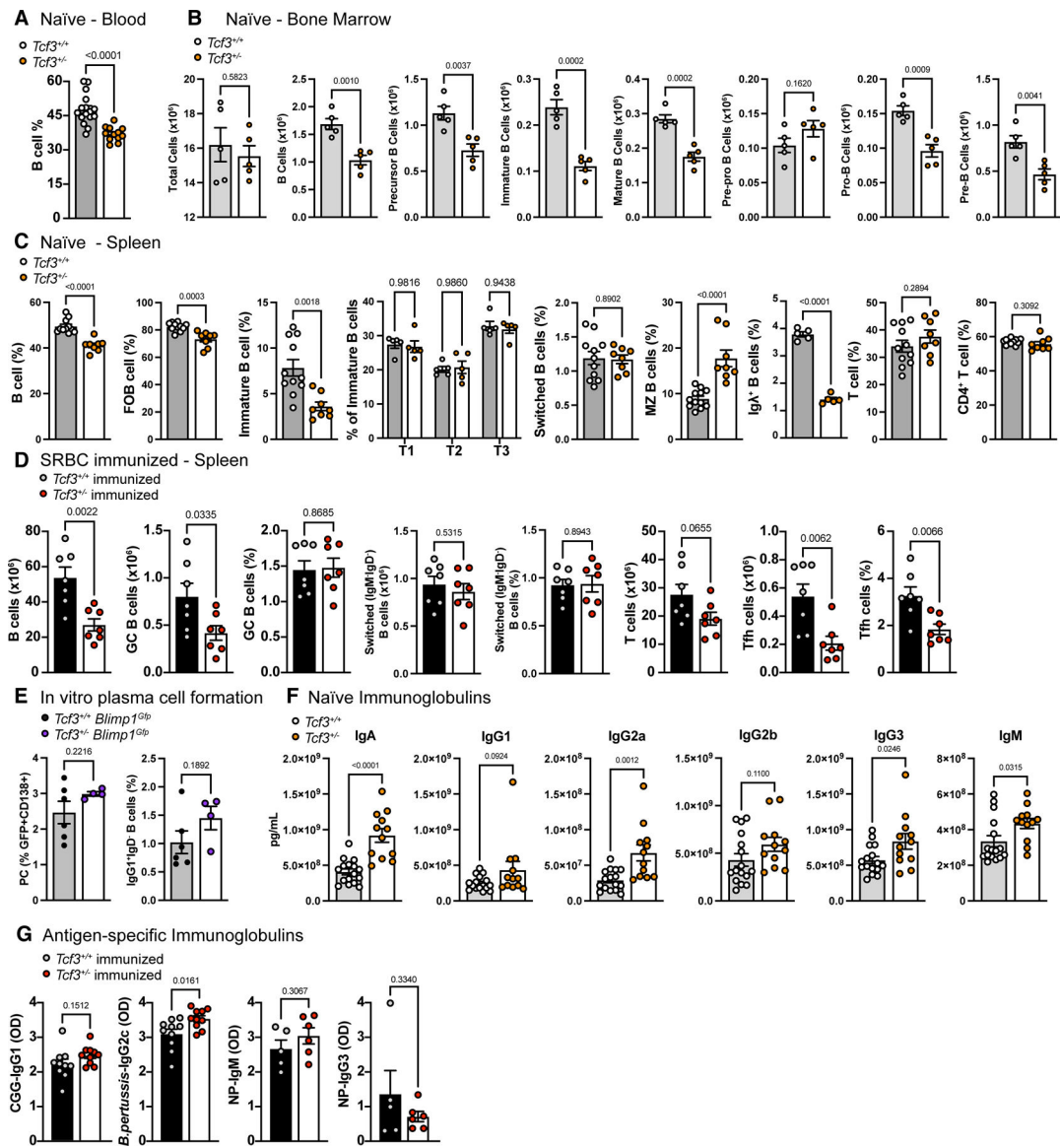
Figure 5:

Haploinsufficiency of TCF3 in mice affects B cell development, but not the humoral immune response. (A) Frequency of total B cells (B220[+]) out of lymphocytes in the peripheral blood of wildtype (*Tcf3^{+/+}*) and heterozygous (*Tcf3^{+/−}*) mice, determined by flow cytometry. (B) Total cell number of B cell populations in the bone marrow of naïve mice, determined by flow cytometry. B cells (B220^{+}), precursor B (B220^{+}IgM^{−}IgD^{−}), immature B (B220^{+}IgM^{+}IgD^{−}), mature B (B220^{+}IgM^{+}IgD^{+}), pre-pro B (precursor, CD43^{+}CD24^{−}), pro-B (precursor, CD43^{+}CD24^{low}), pre-B (precursor, CD43^{−}CD24^{high}). (C) Frequencies of B and T cell populations in the spleen of naïve mice, determined by flow cytometry. B cells (B220^{+}), follicular B cells (FoB; B220^{+}CD93CD23^{+}CD21/35^{low}, immature B (B220^{+}CD93^{+}), transitional 1 (T1; immature B, IgM^{+}CD23^{−}), transitional 2 (T2; (immature B, IgM^{+}CD23^{+}), transitional 3 (T3; immature B, IgM^{low}CD23^{+}), switched B cells (B220^{+}IgM^{−}IgD^{−},), marginal zone B cells (MZ; B220^{+}CD93^{−}CD23^{−}CD21/35^{high}), Igλ^{+}

B cells (B220$^+$Igλ$^+$, shown as % of B cells), T cells (CD3$^+$B220$^-$), CD4$^+$ T cells (T cells, CD4$^+$). (D) Total cell number and frequencies of B and T cell populations of the spleen of sheep red blood cell (SRBC) immunized mice 8 days post immunization, determined by flow cytometry. B cells (B220$^+$), germinal center B cells (GC; B220$^+$GL7$^+$CD95$^+$, shown as total cell number and % of B cells), switched B cells (B220$^+$IgM$^-$IgD$^-$, shown as total cell number and % of B cells), T cells (CD3$^+$B220$^-$), T follicular helper cells (Tfh; CD3$^+$CD4$^+$CXCR5$^+$PD1$^+$, shown as total cell number and % of CD4$^+$ T cells). (E) Frequency of plasma cells (PC; CD19$^+$CD138$^+$GFP$^+$, shown as % of B cells) and class-switched B cells (IgG1$^+$IgD$^-$, shown as % of B cells) in *Tcf3 Blimp1^{Gfp}* reporter mice post in vitro stimulation with LPS, or LPS, IL-4, and IL-5 for 4 days, determined by flow cytometry. (F) Concentration of immunoglobulin isotypes in the plasma of naïve mice, determined by Mesoscale analysis. (G) Optical density (OD) of antigen-specific immunoglobulins in the plasma post immunization, determined by ELISA. Chicken gamma globulin (CGG) and *B. pertussis* antibodies determined 2 weeks post immunization, NP-specific antibodies determined 6 days post immunization. (A-G) Bars represent the mean of the group ± SEM with individual mice represented by colored circles. Statistics show unpaired student's T test assuming for normal distribution and an α of 0.05. (C) Data combined from two independent experiments. (A-E) All populations pre-gated on live, single-cell, lymphocytes.

Laboratory features of individuals with *TCF3* variants.

Table 1:

	Null						HI						DN
	A.II.1	A.II.2	A.II.3	E.II.1	A.I.1	A.I.2	B.II.1	C.II.1	E.I.1	F.I.1	G.I.1	H.II.1	D.II.1
	6y/M	3y/M	8m/F	7y/F	26y/M	23y/F	10y/M	53y/F	32y/F	62y/F	27y/F	6y/F	23y/M
	c.1451–18 A>T	c.1451–18 A>T	c.1451–18 A>T	c.1326+5 G>A	c.1451–18 A>T	c.1451–18 A>T	c.1541 C>A	c.599_600delinsGCT	c.1326+5 G>A	c.1739del	c.1451–18 A>G	chr19: 959646-1649320	c.1663 G>A
product	p.G486Lfs*4	p.G486Lfs*4	p.G486Lfs*4	p.L442_V443ins27	p.G486Lfs*4	p.G486Lfs*4	p.S514*	p.Y200Cfs*54	p.V433*	p.D580Afs*29	p.0	p.0	p.E555K
	Hom	Hom	Hom	Hom	Het	Het	Het	Het	Het	Het	Het	Het	Het
	649 (L)	3723	5398	4081 (H)	2953 (H)	615 (L)	2744 (H)	1007	2368 (H)	1229	2008	2731	1138
	306 (L)	1889	3617	2154	1847 (H)	369	1433 (H)	721	1584 (H)	904	1296	2259	696
	241 (L)	1574 (H)	1430	1633 (H)	937 (H)	204	907 (H)	247	723	283	595	368 (L)	316
	94.1 (H)	80.4 (H)	77	84.5 (H)	89.2 (H)	85.4 (H)	82.9 (H)	70.4	69.6	62.7	73.1	97.2 (H)	90.3 (H)
	44.3	40.8	51.6	44.6	55.8	51.3	43.3	50.4	46.6	45.6	42.9	80.4 (H)	55.4
	34.9 (H)	34 (H)	20.4	33.8	28.3	28.3	27.4	17.3	21.3	13.2	24.2	13.1 (L)	23.4
	1.27	1.2	2.53	1.32	1.97	1.81	1.58	2.91	2.19	3.45	1.77	6.14 (H)	2.2
(DNT)	15.4 (H)	6 (L)	3.2	6.1	4.4	6.4	13.4	3.4	ND	4.6	6.2	3.2	12.1
	16.7 (H)	5.6	5.7	11.7	3.1	5.4	13.4 (H)	ND	ND	6	9.5	1.9 (L)	18.9 (H)
5RA+	22.7 (L)	40.5 (L)	62.6	67.5	22.1	16.6	54.6	14.2	ND	36.2	30.2	44.1	23.8
45RA+	38.5	58.3	78.9	73.7 (H)	46.6	51.1	57.9	30.3	ND	11.8 (L)	2.2 (L)	48.3	50.7
45RA- %)	45.2 (H)	33.7 (H)	18.6	15.4	38.9	39.8	33.5 (H)	54.6	ND	8.9 (L)	2.0 (L)	20.2	36.4
45RA- (%)	15.3	7.5 (L)	2.4 (L)	9.9	13.7	8.6	8.2 (L)	15	ND	36 (H)	61.6 (H)	24.1 (H)	12.6
45RA+	1 (L)	0.5 (L)	0.2 (L)	1 (L)	0.8	0.5	0.4 (L)	0.1	ND	43.3 (H)	34.2 (H)	7.4 (H)	0.3

	Null						HI						DN
45RA+	67.2	61.3	79.5 (H)	42	46.9	66.5	52.1	34.8	ND	4.6 (L)	3.4 (L)	53.4	35.3
45RA– %)	8.5	7.3	7.2	2.2	15.5	6.8	13.8	16.5 (L)	ND	6.8 (L)	1.1 (L)	0.5 (L)	8.4
45RA– (%)	13.7 (L)	14.6	4 (L)	25	25.7	7.6	20.7	13.8	ND	21.7	23.5	4.6 (L)	21.6
45RA+	10.7	16.8	9.4	30.9 (H)	11.9	19.1	13.3	34.8	ND	66.9 (H)	72.0 (H)	41.4 (H)	34.6
5+	7.4 (H)	3.8 (H)	ND	4.1 (H)	2.5	1.7	2.1	ND	ND	7.5	2.2 (L)	5.1 (H)	3.3
XCR5+	3.2 (L)	4.8 (L)	1.8 (L)	3.8 (L)	5.7	7.4	9.2	10.2	ND	3.8	2.8 (L)	3.9 (L)	39.5 (H)
	0 (L)	116 (L)	210 (L)	396	175	50 (L)	136 (L)	124	366	204	166	76 (L)	8 (L)
	0 (L)	2.5 (L)	3 (L)	8.2	5.3	7	4.1 (L)	8.7	10.8	5.7	4.8	2.7 (L)	0.6 (L)
+ ory) (%)	0 (L)	3.1 (L)	3.5	3.4	6.2	9.4	8.1	2.7 (L)	ND	7	10.03	0.1 (L)	0 (L)
7+ emory)	0 (L)	0.6 (L)	0 (L)	1 (L)	3.2 (L)	0.8 (L)	2.7 (L)	1.1 (L)	ND	6.3	6.9	0.1 (L)	0 (L)
D38++ s) (%)	0 (L)	25.7	29.9 (H)	30 (H)	14.3	6.4	8.8	18.3 (H)	ND	10.1	8.3	0.1 (L)	12.2
38++	0 (L)	1.7 (L)	0.4 (L)	0.4 (L)	0.4	0.2 (L)	0.2 (L)	0.3	ND	1.8	1.2	0.1 (L)	0 (L)
	0 (L)	95.6	97.3	98.8	95.9	96	92.3	97.3	ND	80	74.7	89.3	4.8
5+	44 (L)	773 (H)	1388 (H)	333	182	53 (L)	444	297	645	400	314	14	115 (L)
5+ (%)	6.4	16.7	19.8	6.9	5.5 (L)	7.4	13.4	20.8	19	31.1	21.8	0.5	9.1
obulins (mg/dL)													
	137 (L)	295 (L)	230	752#	1262	726	901#	486 (L)	ND	560 (L)	390 (L)	646	33 (L)
	7 (L)	12 (L)	31	27 (L)	312	39 (L)	28 (L)	162	ND	130	70 (L)	43	6 (L)
	32 (L)	11 (L)	12 (L)	17 (L)	45	35 (L)	18.1 (L)	10 (L)	ND	30 (L)	30 (L)	11 (L)	21 (L)
feration & activation													

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	Null					HI					DN
(CD3/	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	ND	Normal
D25)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	ND	Normal
	No B cells	Normal	Normal	ND	Normal	ND	Normal	Normal	Normal	ND	No B cells
	Recurrent respiratory pneumonia	No	No	Bacteremic pneumococcal pneumonia; EBVassociated leukocytosis with	No	Varicella, impetigo, oral thrush, gastroenteritis (not severe)	Recurrent and chronic sinus infection (Mycobacterium avium complex culture +, once)	Recurrent sinusitis, impaired vaccination response to pneumococcus	Pneumonia, bronchitis, vitiligo	Recurrent respiratory infections (RSV)	Gastroenteritis; *Haemophilus influenza* type B meningitis; otitis media; *Rickettsia* infection and pneumonia; bronchiectasis
m	Yes (mild)	Yes (mild)	No	Yes (mild)	No	No	No	No	No	No	No
	Yes	No	No	Yes	No	No	No	No	No	No	No
		Mild intellectual disability					Asthma; granulomatous lung disease; obesity, type 2 diabetes, diabetes	Cholecystectomy	Vitiligo	Crohn's, early onset IBS, Peutz-Jeghers syndrome, mild motor and intellectual developmental delay	

sets are expressed as the percentage among CD4$^+$ or CD8$^+$ T cells, or CD19$^+$ B cells respectively. L and H indicate low and high when compared to age-matched normal ranges.

ults were established through NIH in-house clinical testing; pediatric normal ranges were adopted from Schatorjé et al[35] and Morbach et al.[36]

t therapy. HI, haploinsufficient; DN, dominant negative; Hom, homozygous; Het, heterozygous.