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Molecular basis of common variable immunodeficiency

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Common variable immunodeficiency (CVID) is the most prevalent human primary immunodeficiency requiring medical attention. Until recently, the only known genetic defect specific to CVID was the inducible costimulatory receptor (ICOS) deficiency, which accounts for less than 1% of the patients. Recently, mutations in the TNF receptor family member transmembrane activator and calcium-modulator and cyclophilin ligand interactor (*TACI*), which mediates isotype switching in B cells, were found to be present in 10% to 20% of patients with CVID. Mutations in *TACI* were also found in relatives of patients with CVID who had IgA deficiency (IgAD), as well as in a patient with isolated IgAD. In the majority of patients described to date, only one *TACI* allele is mutated, showing an autosomal dominant transmission of the disease. B cells from individuals with *TACI* mutations did not produce IgG and IgA in response to the *TACI* ligand a proliferation-inducing ligand (APRIL), probably reflecting impaired isotype switching. These results suggest that *TACI* mutations can lead to CVID and IgAD. (*J Allergy Clin Immunol* 2006;117:740-6.)

Key words: Common variable immunodeficiency, IgA deficiency, TNF receptor, B cells, transmembrane activator and calcium-modulator and cyclophilin ligand interactor mutations

Abbreviations used

aa:	Amino acid
AID:	Activation-induced cytidine deaminase
APRIL:	A proliferation-inducing ligand
BAFF:	B-cell activating factor of the TNF family
BCMA:	B-cell maturation antigen
CAML:	Calcium-modulating cyclophilin ligand
CD40L:	CD40 ligand
C _H :	Heavy chain
CRD:	Cysteine-rich domain
CSR:	Class-switch recombination
CVID:	Common variable immunodeficiency
ICOS:	Inducible costimulatory receptor
IgAD:	IgA deficiency
NF-AT:	Nuclear factor of activated T cells
TACI:	Transmembrane activator and calcium-modulator and cyclophilin ligand interactor
TD:	T dependent
TI:	T independent
TNFR:	TNF receptor
TRAF:	TNF receptor-associated factor
WT:	Wild type

THE IMMUNODEFICIENCY: IgA DEFICIENCY AND COMMON VARIABLE IMMUNODEFICIENCY

Common variable immunodeficiency (CVID) is characterized by recurrent bacterial infections and is complicated by autoimmune manifestations in up to 20% of affected individuals and lymphoproliferation with splenomegaly in approximately one third.¹ CVID is diagnosed

on the basis of an impaired ability to produce specific antibodies after vaccination or exposure; markedly reduced serum levels of IgG, IgA, and frequently IgM; and exclusion of other causes for antibody deficiency.² CVID has an estimated prevalence of 1 in 25,000 in white subjects and is the most prevalent human primary immunodeficiency requiring medical attention. Most cases of CVID are sporadic, but at least 10% are familial, with a predominance of autosomal dominant over autosomal recessive inheritance.³ CVID is a complex and heterogeneous disease in which defects in B-cell survival, number of circulating CD27⁺ memory B cells (including IgM⁺CD27⁺ B cells), B-cell activation after antigen receptor cross-linking, T-cell signaling, and cytokine expression have been observed.² The heterogeneity in the clinical symptoms and immunologic defects in CVID might reflect the heterogeneity of the mechanisms leading to the deficiency.

IgA deficiency (IgAD) is the most common form of primary immunodeficiency in the Western world. IgAD is

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characterized by absence or a very low level (<7 mg/dL) of serum IgA. Individuals with IgAD can be asymptomatic or be predisposed to recurrent infections, especially sinopulmonary and gastrointestinal infections. IgAD can be associated with atopy, asthma, and autoimmune disorders.^{4,5} Individuals with symptomatic IgAD might have decreased antibody responses to pneumococcal polysaccharide vaccine, a carbohydrate antigen. Some individuals initially present with IgAD and then develop CVID. IgAD and CVID often coexist in members of the same family. These observations suggest that some cases of IgAD and CVID might have a common cause.

The molecular basis of IgAD and most cases of CVID remains unknown. A small number of individuals diagnosed as CVID have X-linked agammaglobulinemia caused by mutations in Bruton's tyrosine kinase.⁶ A subset of male individuals originally diagnosed as having CVID was found to have mutations in *SH2D1A*, the gene mutated in X-linked lymphoproliferative disorder.⁷ Some individuals with CVID express low levels of CD40 ligand (CD40L), the product of the gene mutated in X-linked hyper-IgM syndrome.⁸ A single report has identified mutations in the inducible costimulatory receptor (*ICOS*) in individuals with CVID who were probably descended from a common founder.⁹ Family studies showed that inheritance varies from an autosomal dominant to an autosomal recessive transmission. Genetic linkage analysis of families with IgAD and CVID identified the presence of susceptibility loci in chromosome 6 within the *MHC* locus near the regions of class I, II, and III.³ The *DR/DQ* locus has been reported to be the strongest predisposing locus. *MHC* class II genes play a fundamental role in antigen presentation to T_H cells that in turn provide help to B cells for a proficient Ig production. Therefore particular *MHC* class II alleles might contribute to the Ig deficiency and to the associated autoimmune manifestations. Other IgAD susceptibility loci located in different chromosomes have been described, although with lower linkage scores.¹⁰ These studies suggest that IgAD and CVID represent a range in the penetrance of the same disease.

Failure to produce IgA in patients with IgAD and Igs in patients with CVID is understood to be due to the failure of B cells to differentiate into a sufficient number of plasma cells. Studies on the molecular events leading to IgA production^{11,12} revealed that some IgA-deficient patients have impaired switching to IgA, whereas others present a postswitch defect.¹²⁻¹⁴ Impaired somatic hypermutation was described in 25% of patients with CVID in one study^{15,16} and in 77% of the patients in another.¹⁷ There is also evidence for a global isotype switching defect in some individuals with CVID.¹⁻³

ISOTYPE SWITCHING IN B CELLS AND DIFFERENTIATION INTO ANTIBODY-PRODUCING PLASMA CELLS

B cells that express and produce Ig derive from IgM^+ B cells that undergo a process of DNA rearrangement called

class-switch recombination (CSR). CSR changes the Ig isotype from IgM to IgG, IgE, or IgA, maintaining the antigen specificity. CSR requires 2 signals.¹⁸ The first is normally delivered by cytokines, which target specific heavy-chain (C_H) genes for transcription, and the second is delivered, in the case of T-dependent (TD) antigens, by interaction of CD40 on B cells with its ligand, CD40L, on activated T cells. The molecular events, common for switching to all isotypes, are C_H germline transcription, induction of activation-induced cytidine deaminase (AID) gene expression, deletional switch recombination, and expression of mature transcripts, which are then translated at high rate by switched B cells differentiating into plasma cells.¹⁸ The specificity of the C_H switch resides at the level of C_H germline transcription.

C_H germline transcription is mainly regulated by signals derived from cytokine-cytokine receptor interactions, such as IL-4-IL-4R and TGF- β -TGF- β R. Cytokines induce the activation and nuclear translocation of specific nuclear factors that activate the C_H promoter of specific C_H genes; for example, TGF- β activates the $C\alpha$ promoter, and IL-4 and IL-13 activate the $C\gamma 1$ and $I\epsilon$ promoters. The C_H promoters contain sequences that can bind transcription factors, such as nuclear factor κB (NF κB), signal transducers and activators of transcription (STATs), B cell-specific transcription factor (BSAP), protein binding a purine-rich box (PU.1), a cellular transcription factor/cyclic AMP-responsive element (ATF/CRE), TGF- β -responsive element (RE), Smads, and activator protein 1 (AP-1).¹⁹⁻²⁵ AID expression is mainly regulated by signals derived from specific ligand-receptor interactions, such as CD40L-CD40, in mice by LPS-Toll-like receptor 4 (TLR4) and in human subjects by CpG-rich oligonucleotides-Toll-like receptor 9 (TLR9).²⁶ The 2 classes of signals, cytokine-cytokine receptor and ligand-receptor signals, synergize in inducing C_H germline transcription and AID expression.

Recently, the TNF family members B-cell activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) have been discovered to induce isotype switching in naive human and mouse B cells.^{27,28} We have shown that BAFF and APRIL induce isotype switching in highly purified IgM^+ B cells derived from CD40-deficient mice and depleted of any residual IgG^+ , IgA^+ , and IgE^+ switched B cells and plasma cells. BAFF and APRIL induced IgG and IgA switching in the absence of exogenous cytokines but were dependent on IL-4 for isotype switching to IgE. At the molecular level, BAFF- and APRIL-induced isotype switching involved C_H germline transcription, AID expression, and deletional switch recombination. Because BAFF and APRIL are expressed primarily on dendritic cells, whereas CD40L is expressed on activated T cells, this novel pathway might underlie T cell-independent isotype switching.

BAFF, APRIL, AND THEIR RECEPTORS

APRIL and BAFF both bind to 2 receptors, B-cell maturation antigen (BCMA) and transmembrane activator

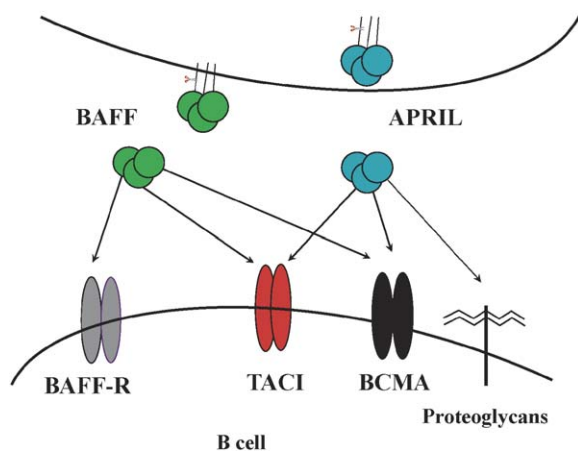


FIG 1. BAFF, APRIL, and their receptors on B cells.

and calcium-modulator and cyclophilin ligand interactor (TACI), which are members of the TNF-R family.²⁹ BCMA is exclusively expressed on B cells, whereas TACI is expressed on B cells and activated T cells. A third receptor, BAFF-R, which is unique for BAFF, is expressed mainly on B cells but also on resting T cells (Fig 1).³⁰ In addition, APRIL, but not BAFF, binds to proteoglycans on the cell surface, including syndecan 1 (CD138), which is highly expressed on plasma cells. This binding is thought to be important for the multimerization of APRIL.^{31,32}

APRIL and BAFF share several features. They (1) are members of the TNF family of ligands, (2) are synthesized as type II transmembrane proteins with a TNF homology C terminal domain, (3) are expressed in monocytes-macrophages and dendritic cells, and (4) are proteolytically cleaved into a soluble form at a multibasic motif by a furin-like protease.³³ BAFF is also highly expressed by neutrophils.³⁴

BAFF enhances B-cell survival. Mice deficient in BAFF have a severe block in B-cell development,^{35,36} very low levels of serum Igs, and impaired specific response to TD and T-independent (TI) antigens. APRIL has no detectable effect on B-cell survival. We have shown that APRIL-deficient mice, in contrast to BAFF^{-/-} mice, have normal B-cell numbers, but their serum IgA levels are diminished, and they have impaired IgA antibody responses to oral immunization with TD antigens and TI antigens.³⁷

BAFF-R is expressed on resting B and T cells and is downregulated on plasma cells. BAFF-R-deficient mice are similar but less severely compromised in their immunophenotype than BAFF^{-/-} mice because they are still able to mount antibody responses against TI antigens, indicating that these might be mediated by TACI.^{38,39} BAFF-R mediates in part isotype switching by BAFF. However, IgA switching in BAFF-stimulated B cells is strictly dependent on TACI.²⁷ The human BAFF-R gene is on chromosome 22.

BCMA is poorly expressed on resting B cells but is upregulated on plasma cells and on germinal center B cells.⁴⁰ BCMA^{-/-} mice have essentially a normal phenotype, although plasma cell survival might be somewhat

impaired.⁴¹ BCMA appears to be involved in antigen presentation.⁴² BCMA plays no detectable role in isotype switching by BAFF and APRIL.²⁷

TACI is expressed on B cells and activated T cells.⁴³ TACI^{-/-} mice have enlarged spleens and lymph nodes with increased cellularity and increased numbers of B cells. In addition, TACI^{-/-} B cells showed an increased proliferation rate and increased Ig production *in vitro*. Despite this *in vitro* B-cell hyperactivity, *in vivo* the serum IgA and IgM levels are low in these mice, and they mount a deficient antibody response to the type II TI antigens Pneumovax (Merck, Whitehouse Station, NJ) and NP-Ficoll (Biosearch Technologies, Novato, Calif). With age, these mice developed autoantibodies with immune complex glomerulonephritis and B-cell lymphoproliferation. There was *in vitro* evidence for decreased apoptosis in the B cells of TACI^{-/-} mice, suggesting that TACI normally delivers an apoptotic signal to B cells and is a regulator of B-cell homeostasis.⁴⁴⁻⁴⁶

The human TACI gene locus is located on the short arm of chromosome 17, which is a common target for mutation and rearrangement. The TACI extracellular N-terminal domain is characterized by 2 cysteine-rich domains (CRDs) that are the hallmark of the TNF receptor (TNFR) superfamily. CRDs contain 6 highly conserved cysteines that generate 3 intrachain disulfide bridges stabilizing the antiparallel β -strands (Fig 2).^{47,48} The first TACI CRD extends from amino acid (aa) 32 to aa 67, which is missing in an isoform of TACI. The second extends from aa 68 to aa 106 and binds APRIL and BAFF with high affinity.⁴⁹ The CRDs contain a conserved 6-residue sequence, (F/Y/W)-D-x-L-(V/T)-(R/G), that is required for APRIL and BAFF binding. TACI, like CD40, recruits TNF receptor-associated factor (TRAF) 2, 3, 5, and 6 and induces NF κ B activation.^{50,51} In addition, TACI induces activation of c-Jun NH2-terminal kinase (JNK) and AP-1. Binding of TRAF2, TRAF3, or both is essential for CD40-mediated activation of nuclear factor κ B and c-Jun NH2-terminal kinase and more importantly for CSR,^{52,53} whereas TRAF6 is important in plasma cell differentiation.⁵⁴ TACI uniquely interacts with calcium-modulating and cyclophilin ligand (CAML), which positively regulates the activation of the calcium-dependent phosphatase calcineurin, which dephosphorylates and activates nuclear factor of activated T cells (NF-AT).^{43,55}

MUTATIONS IN TACI RESULT IN CVID AND IgAD

We and others have recently found that the *TNFRSF13B* (TACI) gene, which encodes TACI, is mutated in a subgroup of patients with CVID.^{56,57} In addition, we identified TACI mutations in a family with IgAD. Our group found 4 different mutations in TACI in 5 of 20 individuals with CVID and 1 of 16 individuals with IgAD. None of these mutations was present in 50 healthy control subjects.⁵⁶ All 6 patients were index cases with other family members found bearing the same mutation. Salzer

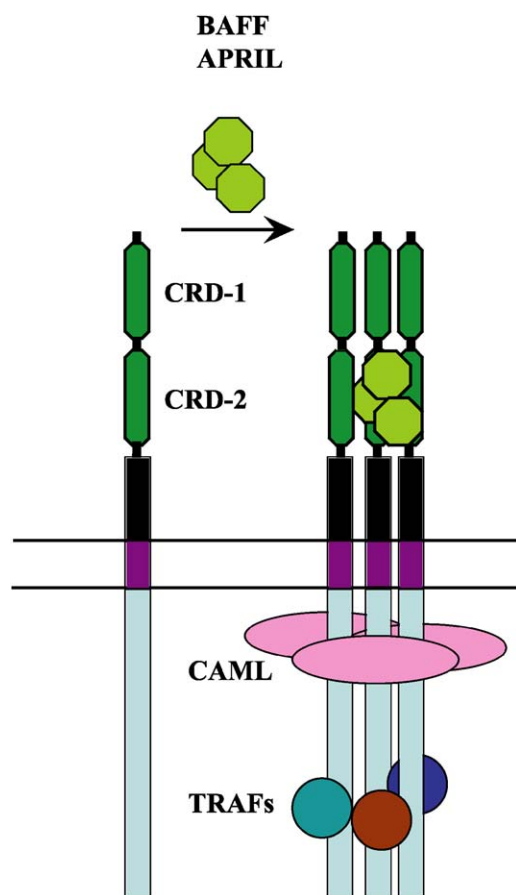


FIG 2. Structure of TACI and ligand-dependent recruitment of intracellular signaling molecules by TACI ligands (BAFF-APRIL).

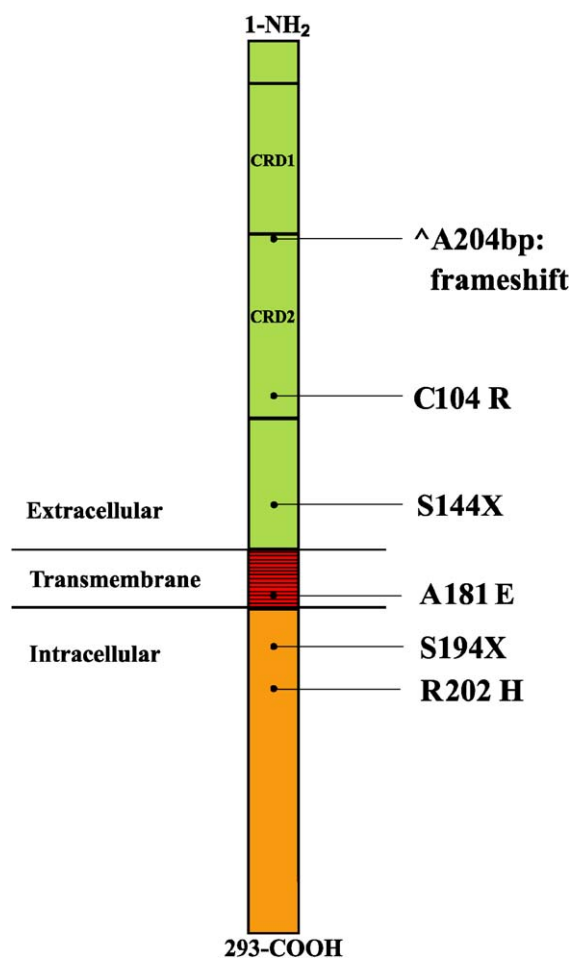


FIG 3. Summary of TACI mutations described to date in CVID.

et al⁵⁷ found 5 mutations in *TACI* in 13 of 162 individuals with CVID. In 3 of the patients' families, other members bearing the same mutation were identified. In total, 6 mutations in the *TACI* gene have been identified to date. The location of the mutations in TACI is shown in Fig 3. The mutations are distributed in all regions of the TACI molecule: 3 in the extracellular domains, 1 in the transmembrane domain, and 2 in the intracellular domains. Three of these mutations are missense mutations, involving a change in one codon that causes a replacement of one aa by another in the protein sequence: cysteine (C)104 to arginine (R), alanine (A)181 to glutamic acid (E), and R202 to histidine (H). Two are nonsense mutations, involving a change in one codon that results in a stop codon: serine (S)144 to stop codon (X) and S194X. One is an insertion of one base, adenine, at the 204th (A204) nucleotide from ATG. This mutation results in a frameshift following aa 68, or 23 in the short isoform of TACI, and premature termination of the protein 11 aa downstream.

Naive B cells from patients with CVID with TACI mutations were severely impaired in their ability to secrete IgG and IgA in response to APRIL *in vitro*.⁵⁶ Although

IgG and IgA secretion by normal naive B cells in this system is likely to represent isotype switching, it is also possible that it might reflect increased survival of already switched contaminating B cells or increased Ig secretion by these cells in response to APRIL stimulation. Because we have previously shown that TACI, but not BCMA, is responsible for IgG and IgA isotype switching and secretion in response to APRIL,²⁷ these results suggest that TACI-mediated signaling was impaired in the patients' B cells.

MECHANISMS OF B-CELL DEFICIENCY IN PATIENTS WITH TACI MUTATIONS

C104 is located in the second CRD of TACI and forms disulfide bonds with the C93 stabilizing the second h2 loop of CRD2.⁴⁹ The C104R mutation was demonstrated to abolish binding of APRIL and BAFF to TACI.^{56,57} In the majority of CVID cases involving the C104R mutation and in the only case of IgAD involving this mutation, only one allele is affected. This raises the question of why patients heterozygous for this mutation do not

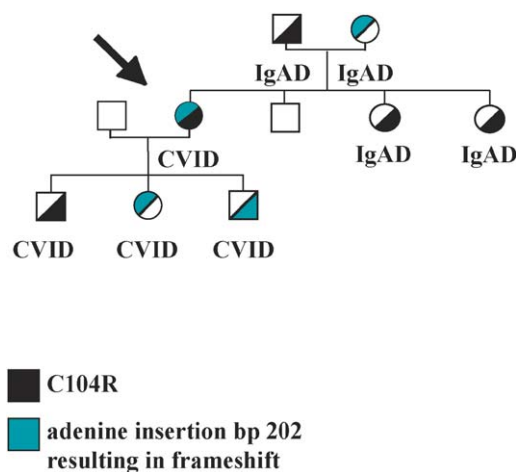


FIG 4. Illustrative family tree of an index patient (arrow) with CVID. Note the presence of both CVID and IgAD in family members with the same TACI genotype.

respond to APRIL and BAFF. The answer might lie in the potential capacity of TNFR family members to undergo ligand-independent preassociation^{58,59} and with the fact that efficient binding of the trimeric ligand might require simultaneous binding to 3 receptor surfaces. Preassociation of wild-type (WT) and C104R mutated TACI would thus behave as a dominant negative and interfere with ligand binding and subsequently ligand-induced signaling. A similar mechanism might explain the B-cell deficiency in patients heterozygous for the A204 insertion because the putative truncated protein might interact with WT protein and interfere with preassembly of TACI molecules. However, the putative truncated protein might not exist because of RNA degradation or protein degradation, as for the S144X mutation.⁵⁷ If this is the case, the explanation is that the expression of a single WT normal allele of *TACI* is not sufficient for effective signaling. On the other hand, mice heterozygous for a null *TACI* allele have normal B-cell function.⁴⁴ However, the phenotype of the *TACI*^{+/-} mice might not reflect the phenotype of patients carrying a heterozygous *TACI* mutation.

S144X leads to premature termination of the protein synthesis. The putative product would not be membrane anchored. The S144X mutation described is homozygous. No TACI protein or mRNA was detected in these patients, suggesting that this mutation confers a susceptibility to RNA and protein degradation. B cells from one such patient were shown not to bind TACI.⁵⁷

A181 is located in the transmembrane domain in proximity of the intracellular portion of TACI. We showed that the A181E mutant is expressed and binds BAFF normally.⁵⁶ A181E results in the substitution of a neutral aa with a negatively charged acidic aa. This might interfere with the capacity of the receptor to aggregate on ligand cross-linking. Alternatively, the mutated protein might be more accessible to proteolytic cleavage or undergo a conformational change that might affect its capacity to signal (eg, by interfering with its ability to interact with CAML and induce NF-AT activation because the

CAML binding site is proximal to the transmembrane domain). Because this mutant is expressed and binds ligand, it might act as a dominant negative by assembling with WT TACI and disrupting signaling.

R202 is in the region involved in CAML binding. The R202H mutant is expressed on transfectants and binds the ligand.⁵⁶ Preliminary data suggest that the R202H mutation might affect the TACI-CAML interaction and thus block TACI-induced NF-AT activation (our unpublished observation). Therefore it might assemble with WT TACI and disrupt signaling by disrupting CAML binding.

The S194X mutation was present in 2 related patients with CVID homozygous for the mutation. The S194X mutation generates a truncated form of TACI in which the region of intracellular domain that contains the docking sequences for TRAF2, TRAF3, and TRAF6 is deleted. Because these TRAF proteins are important for CD40 isotype switching, the S194X mutant would be impaired in its ability to induce isotype switching.

PENETRANCE OF TACI MUTATIONS

In the 4 familial CVID cases that we studied, there was a perfect correlation between the presence of the mutated allele and the occurrence of CVID or IgAD in the family members. In these 4 families, 12 individuals who carried the mutant allele had CVID or IgAD. Eleven of these 12 gave a history of recurrent infections, and 4 were receiving IVIG replacement therapy. In contrast, all 6 individuals who had normal *TACI* genes had normal levels of IgG and IgA and were free of recurrent infections. There was a wide heterogeneity in the severity of the clinical symptoms in the family members with TACI mutations. Symptoms ranged from severe to mild or even nonexistent. There was also a wide variability in serum IgG and IgA levels. In the same family TACI mutation could be associated with CVID or IgAD (Fig 4). In the report by Salzer et al,⁵⁷ 2 brothers with the same homozygous S144X mutation presented with very different phenotypes. This suggests that TACI mutation has different penetrance (ie, the frequency with which a specific phenotype is expressed by individuals with a specific genotype). This might be due to both genetic and environmental modifiers. In this respect mutations in the TNF gene are known to correlate with the severity of CVID.⁶⁰ Furthermore, the MHC locus shows linkage to CVID and IgAD. Thus at least TNF and MHC alleles might influence the penetrance of TACI mutations.

OTHER GENE MUTATIONS IN CVID

Mutations in ICOS have been found in 1% of patients with CVID analyzed.⁹ ICOS is a costimulatory T-cell molecule that strongly induces IL-10 production and also increases the secretion of IL-4, IL-5, and IL-6. These cytokines are produced by T_H2 T cells important in T-cell/B-cell cooperation for CSR, Ig production, and

terminal differentiation of B cells into memory cells and plasma cells. Patients bearing mutations in the *ICOS* gene, like *ICOS* and *ICOS-L* knockout mice, show a defect in germinal center formation and impaired humoral immune responses.^{9,61-65}

Homozygous CD19 mutations have been reported in 2 families with CVID.⁶⁰ CD19 regulates the signaling for B-lymphocyte development, activation, and proliferation. In mice CD19 deficiency results in hypogammaglobulinemia, defect in germinal center formation, and impaired antibody responses to T cell-dependent antigens.^{66,67} Another member of the TNFR family, BAFF-R, has been found to be mutated in a patient with CVID and an unaffected relative.⁵⁸ BAFF-R is important for B-cell development and survival. In mice a defect in BAFF-R-BAFF interaction leads to a hypogammaglobulinemia defect in germinal center formation and impaired antibody responses to T cell-dependent antigens.^{38,39,68} The fact that the mutation in CD19 and BAFF-R deficiency affected both alleles suggests autosomal recessive transmission.

CONCLUSION AND THERAPEUTIC IMPLICATIONS

In summary, TACI mutations leading to an immunodeficiency account for 10% to 15% of the cases of CVID and also for about 5% of patients with IgAD, the highest incidence of a monogenic defect ever found in IgAD and CVID. Moreover, the genetic transmission in most of the patients with CVID with a TACI mutation is autosomal dominant, with variable penetrance in family members carrying the mutated allele. These findings should allow accurate genetic diagnosis in a subgroup of patients with CVID-IgAD and their relatives. It will remain to be seen whether useful genotype-phenotype correlations can be drawn in patients with TACI mutations regarding severity of infections, development of granulomas, autoimmunity, and malignancy.

TACI normally delivers an apoptotic signal to B cells, as evidenced by *in vitro* studies and by the development of autoimmunity and B-cell lymphoproliferation in TACI-deficient mice. Defective TACI signaling in patients with CVID and IgAD might underlie the susceptibility of these patients to have autoantibodies, benign lymphoproliferation with splenomegaly, and malignant lymphoma and nonlymphoid malignancies. B-cell malignancy in these patients might relate to the apoptotic signal normally delivered by TACI. Finally, the fact that TACI is predominantly expressed in B cells suggests that autoimmunity and B-cell malignancy might develop in a B cell-autonomous fashion in patient with TACI mutation. This raises the possibility that anti-CD20 mAb might be of value in patients with TACI mutations who experience life-threatening autoimmune disease or B-cell lymphoma.

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