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Functional analysis of TACI mutations associated with CVID

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Capsule Summary

Some, but not all, TACI mutations found in CVID impair TACI function and may potentially contribute to B cell dysfunction in heterozygotes via haploinsufficiency.

Keywords

TACI; common variable immunodeficiency; APRIL; BAFF; BLyS

To the Editor

Mutations in *TNFRSF13B*, the gene encoding for the transmembrane activator, calcium-modulator and cyclophilin ligand interactor (TACI), have been identified in 5–10% of patients with CVID ^{1, 2}. The most common TACI variants in CVID are C104R, in the ligand binding cysteine-rich domain 2 (CRD2), and A181E, in the transmembrane (TM) domain of TACI. Each is found in the heterozygous state in 2–5% of CVID patients, but also in 0.5–1% of healthy individuals ^{1–3}. Both destroy the capacity of TACI ligation to activate NFκB. We have examined the function of TACI coding variants that have been described in CVID patients and their relatives, but so far not in healthy individuals ^{3–5}.

Figure 1A shows the location of the missense mutations examined in the TACI protein. The CRD1 mutants W40R and D41H, the CRD2 mutant I87N, the TM domain mutants C172Y and A181E, and the IC domain mutants K188M and V246F were expressed on the surface of 293T cell transfectants at levels comparable to WT TACI (Figure 1B). Surface expression of the CRD2 mutants Y79C and C104R was modestly decreased to ~ half of WT, while that of the L171R mutant was reduced ~8 fold. Protein expression of the L171R mutant in whole cell lysates was similarly reduced, but its mRNA expression was comparable to that of WT (data not shown), suggesting that the mutant protein was unstable. Both of the CRD1 domain mutants, W40R and D41H, showed normal binding of BAFF (Fig. 1C). There was total loss of BAFF binding by the Y79C and C104R mutants. The I87N mutant had residual BAFF binding, which increased with increasing concentrations of ligand (Fig. S1). The poorly expressed L171R mutant had negligible ligand binding. The TM domain mutants, C172Y and A181E and the IC domain mutants K188M and V246F had intact BAFF binding.

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TACI signaling was examined by measuring NFκB and NF-AT activation using a dual luciferase reporter system⁶. Mutant or WT TACI were transfected into 293T cells with either NFκB luciferase or NF-AT luciferase reporter plasmids together with Renilla luciferase, and the capacity of APRIL (50 ng/ml) to induce reporter gene activity was determined. The CRD1 domain mutants (W40R, D41H) activated NFκB and NF-AT at levels comparable to WT TACI (Fig. 2A and B). The Y79C and C104R mutants failed to activate either NFκB or NF-AT, consistent with their failure to bind BAFF. The I87N mutant demonstrated residual reporter gene activation. Increasing the concentration of APRIL resulted in increased NFκB activation in I87N transfected cells (Fig. S2), consistent with the decreased ligand binding affinity of this mutant. Ligand-induced activation of NFκB and NF-AT was abrogated in the TM domain mutant L171R, likely a consequence of its markedly diminished surface expression. Despite normal ability to bind ligand, the TM mutants C172Y and A181E failed to signal. Signaling by the IC domain mutants (K188M, V246F) was intact.

Since TACI assembles as a trimer or higher order oligomer⁶, TACI mutants with disrupted function might potentially exert a dominant negative effect on signaling by WT TACI in heterozygotes. 293T cells were co-transfected with equal amounts of WT TACI and either empty vector or TACI mutant cDNA and ligand driven NFκB activity was measured. Results were calculated as the percentage of the net increase in NFκB activity in cells transfected with WT TACI and empty vector. None of the mutants analyzed exhibited a significant inhibition of NFκB activation by WT TACI (Fig. 2C). The absence of a DN effect by the C104R mutant is consistent with our recent demonstration that introduction of a murine TACI transgene, which carries the C76R TACI mutation equivalent to the human C104R mutation, into the TACI^{+/-} background does not exert a DN effect on TACI function⁷.

The data obtained on the mutants included in our study is summarized in Table 1. The mutations that impaired TACI function did not exert a DN effect, but could cause B cell dysfunction in heterozygotes due to haploinsufficiency. This is supported by the observation that CVID patients who are heterozygous for C104R have poor *in vitro* responses to APRIL^{1, 2}. It remains to be addressed whether healthy subjects with this and other TACI mutations that affect TACI function have TACI haploinsufficiency that remains clinically silent. Deficiency in the TACI homologue BAFF-R has been reported to result in poor antibody responses to pneumococcal cell wall polysaccharides in two siblings who carry the same BAFF-R mutation, but caused CVID in only one of the siblings⁸. Potential synergy of TACI haploinsufficiency with other defects, such as defective B cell responsiveness to TLR9^{9, 10}, which is important for memory B cell formation, might determine its penetrance and needs to be investigated in CVID patients with TACI mutations.

The absence of a DN effect of deleterious TACI mutants on signaling by WT TACI is unlikely the result of their failure to assemble with WT TACI, as the C104R and A181E mutants assemble normally with WT TACI⁶ (and data not shown). We previously suggested that the C104R mutant might exert a DN effect, based on calculations of fold induction of NFκB activity in cells co-transfected with WT and mutant TACI compared to cells co-transfected with WT TACI and empty vector⁶. However, re-evaluation of the data indicates that the absolute increase in NFκB activity was comparable in both co-transfectants, but that background NFκB activity in the cells co-transfected with WT and mutant TACI was ~ double that of cells transfected with WT TACI and empty vector. This increased background was responsible for the observed reduction in fold induction, and is likely due to the fact that the mutant preassembles and constitutively signals comparably to WT TACI⁶.

The use of transfectants to examine the expression and function of mutant proteins has limitations, because the unphysiologic levels of expression of the mutant protein in this system may mask subtle alterations of protein expression, and because it does not reveal the impact of the mutants on B cell function in a physiologic setting. This is best achieved by examining B cell function in patients, their healthy relatives, and unrelated individuals who carry the same mutations, and by the study of transgenic mice that carry the mutant allele on a homogeneous background.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

APRIL	A Proliferation-Inducing Ligand
BAFF	B Cell Activating Factor of the TNF family
CAML	Calcium-modulating cyclophilin ligand
CRD	Cysteine rich domain
CVID	Common variable immunodeficiency
DN	Dominant negative
EC	Extracellular
IC	Intracellular
NFAT	Nuclear factor of activated T cells
NFκB	Nuclear factor kappa B
TACI	Transmembrane activator and CAML interactor
TM	Transmembrane
TRAF	TNF Receptor Associated Factor

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Figure 1. TACI mutants: Location, expression and ligand binding

A. Location of the mutated a.a. in the TACI structure. **B, C.** FACS analysis of TACI expression (B) and BAFF binding (C) by 293T cells transfectants. Similar data was obtained in three independent experiments. Net mean fluorescence intensity (MFI) values for TACI expression, calculated as the MFI of transfectants minus that of empty vector controls: WT 213 ± 23 , W40R 206 ± 18 , D41H 201 ± 19 , Y79C 110 ± 7 , I87N 227 ± 17 , C104R 123 ± 8 , L171R 26 ± 3 , C172Y 197 ± 36 , A181E 190 ± 9 , K188M 222 ± 19 , V246F 226 ± 28 ($n=3$; $p < 0.05$ for Y79C, C104R and L171R and $p > 0.05$ for all other mutants).

Figure 2. NFκB and NFAT activation induced by APRIL binding to TACI-transfected 293T cells
A, B. NFκB- (A) and NFAT- (B) driven luciferase activity in 293T cells transfected with WT and TACI mutants. Each of the mutants was examined in parallel with WT TACI in three independent experiments and the results are expressed as a mean percentage of the ligand induced luciferase activity in 293T cells transfected with WT TACI. **C.** Effect of co-transfection of TACI mutants on APRIL-driven NFκB activation by WT TACI. Results are expressed as a percentage of the ligand induced luciferase activity in 293T cells co-transfected with WT TACI and empty vector. Columns and bars represent mean and SD of three independent experiments.

Table 1

Summary of the effect of TACI mutations on TACI expression and function

Mutation	Domain	Expression	Ligand binding	κB/NFAT NF signaling	Dominant negative effect
W40R	CRD1	Normal	Normal	Normal	N/A
D41H	CRD1	Normal	Normal	Normal	N/A
Y79C	CRD2	Modestly decreased	Absent	Absent	No
I87N	CRD2	Normal	Impaired	Impaired	No
C104R	CRD2	Modestly Decreased	Absent	Absent	No
L171R	TM	Strongly decreased	Absent	Absent	No
C172Y	TM	Normal	Normal	Absent	No
A181E	TM	Normal	Normal	Absent	No
K188M	IC	Normal	Normal	Normal	N/A
V246F	IC	Normal	Normal	Normal	N/A

N/A = Not applicable. These mutations do not affect TACI function