Transmembrane activator and CAML interactor *(TACI)* haploinsufficiency results in B-cell dysfunction in patients with Smith-Magenis syndrome

Javier Chinen, MD, PhD,[a]* Monica Martinez-Gallo, PhD,[b]*‡ Wenli Gu, PhD,[c] Montserrat Cols, PhD,[b] Andrea Cerutti, MD,[b] Lin Radigan,[b] Li Zhang, PhD,[b] Lorraine Potocki, MD,[c] Marjorie Withers, MS,[c] James R. Lupski, MD, PhD,[a,c] and Charlotte Cunningham-Rundles, MD, PhD[b] *Houston, Tex, and New York, NY*

Background: Heterozygous deleterious mutations in the gene encoding the tumor necrosis factor receptor superfamily member 13b *(TNFRSF13B)*, or transmembrane activator and CAML interactor *(TACI)*, have been associated with the development of common variable immunodeficiency. Smith-Magenis syndrome (SMS) is a genetic disorder characterized by developmental delay, behavioral disturbances, craniofacial anomalies, and recurrent respiratory tract infections. Eighty percent of subjects have a chromosome 17p11.2 microdeletion, which includes *TACI*. The remaining subjects have mutations sparing this gene.

Objective: We examined TACI protein expression and function in patients with SMS to define the role of *TACI* haploinsufficiency in B-cell function.

Methods: We studied *TACI* expression and function in a cohort of 29 patients with SMS.

Results: In patients with SMS with only 1 *TACI* allele, we found decreased B-cell extracellular and intracellular expression of TACI, reduced binding of a proliferation-inducing ligand, and decreased TACI-induced expression of activation-induced cytidine deaminase mRNA, but these were normal for cells from patients with SMS and 2 *TACI* alleles. Impaired upregulation of B-cell surface TACI expression by a Toll-like receptor 9 agonist was also observed in cells from patients with 1 *TACI* allele. Gene sequence analysis of the remaining *TACI* allele revealed common polymorphisms, with the exception of 1 patient with an amino acid change of uncertain significance. Patients with SMS with the lowest TACI expression had significantly reduced antibody responses to pneumococcal vaccine serotypes.

Discussion: Our findings suggest that haploinsufficiency of the *TACI* gene results in humoral immune dysfunction, highlighting the role of genomic copy number variants in complex traits. (J Allergy Clin Immunol 2011;127:1579-86.)

Key words: *B cell, humoral immunity, transmembrane activator and CAML interactor, common variable immunodeficiency, Smith-Magenis syndrome, gene haploinsufficiency*

The tumor necrosis factor receptor superfamily member 13b *(TNFRSF13B)*, also known as transmembrane activator and CAML interactor (TACI), is a cell-membrane receptor for the ligands a proliferation-inducing ligand (APRIL; TNFSF13) and B cell–activating factor (TNFSF13B).[1] TACI activation of B cells leads to their differentiation and maturation, including antibody isotype switch,[2-6] and T cell–independent antibody production.[7] Mutations in *TACI* have been found in 8% to 10% of patients with common variable immunodeficiency (CVID),[8-12] suggesting a role for these mutations in the development of low serum immunoglobulin levels and lack of antibody characteristic of this disorder. The mutations found in the immunodeficient subjects appeared to be inherited in a dominant fashion in some cases, suggesting that the mutant protein, even in the heterozygous state usually found in patients, might hinder or abolish the function of the trimeric signaling complex. Alternatively, the mutant protein could lead to insufficient expression of a functional complex with stringent ligand-binding requirements.[6] Homozygous and heterozygous *TACI*-null mutations have been described in a few cases of CVID.[8,11,12] In one of these families, reduced expression of the normal allele in heterozygous relatives was noted,[8] suggesting that the pathogenesis of B-cell dysfunction might include *TACI* haploinsufficiency.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation disorder estimated to occur in 1:15,000 to 1:25,000 persons and is most commonly associated with an approximately 3.7-Mb interstitial deletion within chromosome 17p11.2 (>80% to 90% of patients) or rarely with a point mutation in the retinoic acid–induced gene 1 *(RAI1)*, which is viewed as the genetic cause of this syndrome.[13-15] The clinical features include developmental delay; neurobehavioral abnormalities, including sleep disturbances; and craniofacial and other skeletal anomalies. Eighty-eight percent of a cohort of 44 patients with the 17p11.2 deletion were noted to have chronic ear infections, whereas 6 (60%) of 10 patients with SMS with point mutations in *RAI1* reported these infections.[16,17] *TACI* maps within the common SMS deletion in chromosome 17p11.2, and therefore most patients with SMS are heterozygous null and haploinsufficient for this gene. Although immune defects have not been

From [a]the Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston; [b]the Immunology Institute and the Department of Medicine, Mount Sinai Medical Center, New York; and [c]the Department of Human and Molecular Genetics, Baylor College of Medicine, Houston.

*These authors contributed equally to this work.

‡Dr Martinez-Gallo is currently affiliated with the Vall d'Hebron University Hospital, Barcelona, Spain.

Supported by National Institutes of Health grants AI-101093, AI-467320, and AI-48693 and National Institute of Allergy and Infectious Diseases Contract 03-22 (C.C.-R.).

Disclosure of potential conflict of interest: J. R. Lupski is a consultant and on the speakers' bureau for Athena Diagnostics. The rest of the authors have declared that they have no conflict of interest.

Received for publication September 10, 2010; revised January 6, 2011; accepted for publication February 8, 2011.

Available online April 22, 2011.

Reprint requests: Charlotte Cunningham-Rundles, MD, PhD, Departments of Medicine and Pediatrics, The Immunology Institute–Mount Sinai School of Medicine, 1425 Madison Ave, New York, NY 10029. E-mail: Charlotte.Cunningham-Rundles@mssm.edu.

0091-6749/$36.00

doi:10.1016/j.jaci.2011.02.046

Abbreviations used
AICD: Activation-induced cytosine deaminase
APRIL: A proliferation-inducing ligand
CVID: Common variable immunodeficiency
MFI: Mean fluorescence intensity
PE: Phycoerythrin
RAI1: Retinoic acid–induced gene 1
SMS: Smith-Magenis syndrome
TACI: Transmembrane activator and CAML interactor
TLR: Toll-like receptor
TNFRSF13B: TNF receptor superfamily member 13b

explored in patients with SMS, we hypothesized that the loss of 1 *TACI* allele would lead to deficient expression of the TACI receptor and would allow us to examine the significance of this protein on the humoral immune function in a cohort of patients with SMS.

METHODS

Patients and control subjects

Patients with a molecularly established SMS diagnosis who were enrolled in an institutional review board–approved protocol at Baylor College of Medicine were studied. SMS diagnosis was confirmed by means of detection of an interstitial deletion of chromosome 17p11.2 that includes the *RAI1* gene by using fluorescence *in situ* hybridization or by a mutation in the *RAI1* gene identified by means of direct sequencing. For this study, 25 patients with SMS with the common deletion including *TACI* were compared with 4 others in whom *TACI* was not deleted. The clinical history was reviewed, and serum IgG, IgA, and IgM levels and antibody concentrations to 12 pneumococcal serotypes were determined by means of ELISA (IBT Technologies, Kansas City, Kan). Protective levels of 1 μg/mL were used for each serotype. Patients' demographics are presented in Table I. EBV-transformed B-cell lines were produced from the patients' peripheral blood. For comparison, EBV-transformed cell lines of healthy control subjects with and without known mutations involving *TACI* were examined.

Extracellular and intracellular TACI protein expression

Surface expression of TACI on EBV-transformed B-cell lines from patients with SMS and healthy control subjects was determined by means of flow cytometric assays using biotinylated polyclonal goat anti-TACI (1:400) antibody or a control biotinylated goat IgG and secondary staining with 1:200 streptavidin-phycoerythrin (PE; BD PharMingen, San Jose, Calif). Intracellular TACI expression was determined by using goat anti-TACI (1:200) antibody after treatment with permeabilization reagents and followed by streptavidin-PE staining.

Laser-scanning confocal microscopy

B-cell lines were resuspended in Cell Adhesive Solution, as instructed by the manufacturer (Crystalgen, Inc, Commack, NY), and then were applied to slides (Gold Seal; Thermo Scientific, Portmouth, NH), fixed with 1.6% (vol/vol) paraformaldehyde, and made permeable with 0.2% (vol/vol) Triton X-100 in PBS. Cells were stained with murine anti-TACI mAb (clone 11H3; eBioscience, San Diego, Calif). Negative controls were first stained with unconjugated murine antibody with irrelevant binding activity (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif). All slides were then stained with secondary antibodies (donkey anti-mouse IgG AlexaFluor-488; Molecular Probes, Invitrogen, Inc, Carlsbad, Calif). Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole; Boehringer Mannheim, Indianapolis, Ind). Slides were covered with cover slips through the use of FluorSave reagent (Calbiochem, San Diego, Calif) and examined as previously described.[18]

Ligand APRIL binding

EBV-transformed B-cell lines were cultured at 37°C in RPMI 1640 medium with L-glutamine and 10% heat-inactivated FCS in triplicates. To assess binding of the ligand APRIL, B cells were incubated with 250 ng/mL FLAG-tagged megaAPRIL (Axxora, San Diego, Calif) on ice in the presence of heparin (1000 U/mL); 1 μg/mL biotin-anti FLAG monoclonal M2 antibody (Sigma, St Louis, Mo) was then added. The cells were washed and examined with streptavidin-PE. Flow cytometry (FACS) was performed with a FACSCalibur (Becton Dickinson, Mountain View, Calif).

Analysis of *TACI* isoforms mRNA

Total RNA was extracted from B-cell lines, and cDNA was prepared with a SuperScript first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, Calif). First-strand cDNA was synthesized with 100 ng of total RNA in a 20-μL reaction mixture by using Invitrogen's protocol. PCR amplification was performed with previously published primers covering exons 1 to 3 and exons 3 to 5.[12,15] The exon 3 to 5 amplicon was used to measure total *TACI* mRNA expression by means of quantitative PCR. Briefly, a 50-μL reaction mixture was prepared with 5 μL of 10× PCR buffer, 0.5 μL of 25 mmol/L $MgCl_2$, 1.0 μL of deoxynucleotide triphosphates (10 mmol/L), 10 pmol of each primer, and 0.25 units of Hot-Taq DNA polymerase (Qiagen, Valencia, Calif). The PCRs were run under the following conditions: 95°C for 15 seconds, 63°C for 30 seconds, and 72°C for 1 minute and 30 seconds for 30 cycles. The PCR product was finally visualized on agarose gel electrophoresis containing ethidium bromide. Quantitative RT-PCR was conducted with a LightCycler SYBR Green I Detection System (Roche Diagnostics, Indianapolis, Ind). For this, 2-μL samples (cDNA) were run in duplicates for glass capillary reaction tubes in a total volume of 20 μL. The RT-PCR products of gene expression were determined copy number per microgram of RNA relative to β-actin. The β-actin primers used were as follows: forward, 5′-CCC CCT GAA CCC CAA GGC CAA CCG CGA GAA-3′; reverse, 5′-TAG CCG CGC TCG GTG AGG ATC TTC ATG AGG-3′.

TACI-induced expression of activation-induced deaminase mRNA

Fifty thousand EBV-transformed B cells were cultured in 48-well plates with an agonistic monoclonal TACI (clone 174; R&D Systems, Inc, Minneapolis, Minn) or isotype control with or without 100 ng/mL IL-4 (R&D Systems, Inc) to upregulate activation-induced deaminase *(AICD)* mRNA.[19] Five milliliters of goat anti-mouse IgG microbeads (Miltenyi Biotec, Carlsbad, Calif) was added to further crosslink anti-TACI. After 48 hours, mRNA was isolated (RNeasy Mini Kit; Qiagen, Valencia, Calif) and reverse transcribed (SuperScript III First-Strand cDNA synthesis kit, Invitrogen). Quantitative real-time PCR was performed with the LightCycler Real Time PCR system and the FastStart DNA Master SYBR Green I kit (Roche Diagnostics), with β-actin mRNA as a control for cell copy number. The following primers were used: *AICD* (5′-TGCTCTTCCTCGGCTACATCTC-3′; 5′-AACCTCATACAGGGGCAAAAGG-3′) and β-actin (5′-CTGAACCC-CAAGGCCAACAG-3′; 5′-CCAGAGAAGAGGAGGATGCG-3′).

Surface TACI expression by Toll-like receptor stimulation

Stimulation of peripheral blood B cells by Toll-like receptor (TLR) 9 ligation enhances the expression of TACI on normal B cells. To determine whether this was possible in cells from patients with SMS, we examined the B cells of 2 patients with the common deletion, incubating peripheral blood B cells with CpG-DNA and oligonucleotide ODN2006 at 0, 0.6, 1.5, and 3.0 μg/mL