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Abatacept alleviates severe autoimmune symptoms in a patient carrying a *de novo* variant in *CTLA-4*

To the Editor:

Inhibitory immune receptors presented on regulatory T (Treg) cells provide a critical balance in immune modulation by interacting with ligands expressed in antigen-presenting cells. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) on Treg cells suppresses immune reactions by interacting with CD80 and CD86, which are displayed by antigen-presenting cells, making it a useful target for immune modulation. Haploinsufficiency of *CTLA-4* in human or knockout of the gene in mouse leads to immune dysregulation.[1-3] Here we describe a patient who presented with severe multiorgan autoimmune disorders. Genetic analysis of the patient genome revealed a functional mutation in *CTLA-4*, and treatment with CTLA-4-Ig agent, a CTLA-4 mimetic, reconstructed Treg-cell functionality and improved the patient's condition, suggesting CTLA-4-Ig agent as a potential therapeutic solution for autoimmune patients with *CTLA-4* mutation.

The patient is a 14-year-old Korean girl who has suffered from chronic diarrhea since age 3 months (Fig 1, *A*, and see Fig E1 in this article's Online Repository at www.jacionline.org). At the age of 4 years, esophagogastroduodenoscopy with biopsy revealed villi atrophy and heavy lymphoplasma cell infiltration in the lamina propria of the duodenum, leading to the diagnosis of autoimmune enteropathy (see Fig E2 in this article's Online Repository at www.jacionline.org). At the age of 7 years, she developed anemia with vitamin B_{12} deficiency. Autoimmune hepatitis was diagnosed at the age of 8 years with periodically high levels of AST (aspartate transaminase, >1000 IU/L) and ALT (alanine transaminase, >1000 IU/L) and liver biopsy. Antiplatelet antibody and smooth muscle antibody signal turned positive, and transiently elevated IgG level was noted (see Table E1 in this article's Online Repository at www.jacionline.org). At the age of 11 years, the patient was admitted with high fever (39°C) and jaundice accompanied by pancytopenia. Direct Coombs test result was positive. Bone marrow examination for the evaluation of pancytopenia revealed hemophagocytosis, and corticosteroids and antibiotics were applied. Subsequently, pulmonary aspergillosis and sepsis were observed. At age 12 years, she developed hematochezia and endoscopic evaluation revealed mixed tubular adenocarcinoma of the stomach (stage 1, T3, N0; see Fig E3 in this article's Online Repository at www.jacionline.org). She underwent a subtotal gastrectomy with surgical liver wedge biopsy. Hepatic pathology showed micronodular liver cirrhosis. Multiple areas of joint pain were noted before the surgery. She remained below the third percentile of Korean growth references for height and weight and virtually stopped growing after the surgery (see Fig E4 in this article's Online Repository at www.jacionline.org). Multiple rounds of immunosuppressants were administrated during the course, but none achieved substantial improvements (see Fig E1). The patient does not carry any notable protein-altering variants in *FOXP3* or *AIRE* by Sanger sequencing, excluding the possibility of suffering from these genetically predefined autoimmune syndromes.

Whole-exome sequencing of the patient and her healthy parents led to the discovery of a nonsynonymous *de novo* variant (p.Pro138Thr) in *CTLA-4* (Fig 1, *B*; see Tables E2, E3, and E5 and Figs E5-E8 in this article's Online Repository at www.jacionline.org). The point mutation lies in the extracellular MYPPPY ligand-binding motif (Fig 1, *C*).[4] The motif is well conserved in most of the vertebrates, and the mutated residue Pro138 is completely conserved in all the vertebrate species examined. Structural analysis demonstrated that the motif forms the ligand interacting surface for CD80 and CD86 (Fig 1, *D*; see Fig E9 in this article's Online Repository at www.jacionline.org).[5,6] Reconstructing T-cell stimulation *in vitro* demonstrated that the expression of wild-type CTLA-4 in Jurkat cells successfully implemented the immune modulation activity as assayed by IL-2 production while the introduction of the mutation into CTLA-4 eliminated the immune modulation activity (Fig 1, *E*).

Next, we tested whether controlling the CTLA-4–mediated signal through medication could improve the patient's condition. Abatacept (Orencia) is a fusion protein formed by an IgG_1 Fc region linked with the extracellular domain of CTLA-4 (CTLA-4-Ig), and is commonly used to alleviate rheumatoid arthritis symptoms. Notably, there was a recent case report on abatacept treatment in an adult with idiopathic autoimmune enteropathy.[7] To investigate the molecular effect of the drug, we sampled the patient's blood at the trough level (ie, 0 day) and at 1, 4, 6, and 8 days (among these, samples from the trough level and days 4 and 8 were subjected to the fluorescence-activated cell sorting analysis) after the administration of the drug (Fig 2, *A-D*; for drug treatment details, see the Methods section in this article's Online Repository at www.jacionline.org). Notably, the proportion of $CD25^+$ Treg cells ($CD4^+CD25^+FOXP3^+$ or $CD4^+CD25^{high}CD127^-$ cells) was lower in the patient than in healthy donors while the proportion of $CD4^+FOXP3^+$ cells was not reduced in the patient when compared with healthy donors, as appeared in the previous report on patients with *CTLA-4* haploinsufficiency (Fig 2, *A*; see Fig E10 in this article's Online Repository at www.jacionline.org).[1] Moreover, administration of the

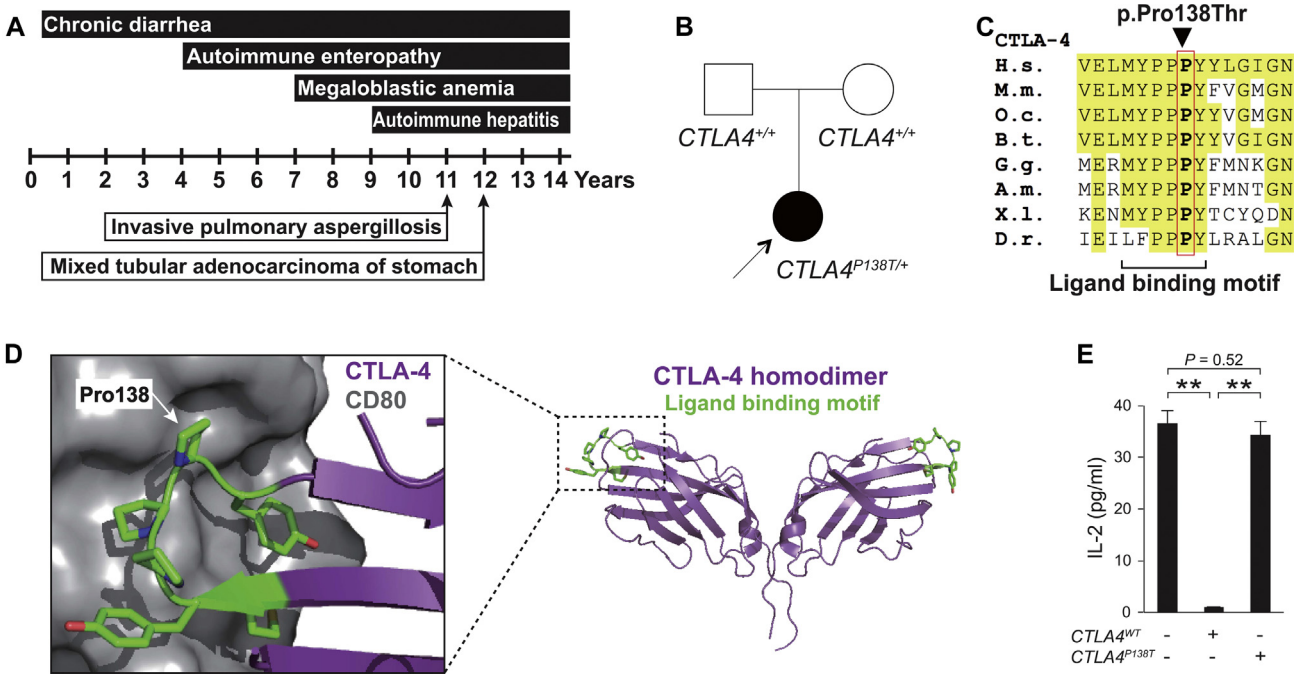

FIG 1. *De novo CTLA-4* mutation in early-onset multiorgan autoimmune disease. **A** and **B,** Disease course and pedigree of the patient. **C,** The evolutionary conservation of the mutated reside. **D,** Structure of CTLA-4 homodimer *(right)*[5] and interaction with CD80 *(left)*.[6] **E,** Overexpressed CTLA4^{P138T} lost the ability to suppress IL-2 production in Jurkat cells. *A.m.,* Alligator mississippiensis; *B.t.,* Bos taurus; *D.r.,* Danio rerio; *G.g.,* Gallus gallus; *H.s.,* Homo sapiens; *M.m.,* Mus musculus; *O.c.,* Oryctolagus cuniculus; *X.l.,* Xenopus laevis. $^{**}P < .005$.

drug actively reduced CD25 expression and increased FOXP3 expression as indicated by the proportion of CD4$^+$FOXP3$^+$ cells (Fig 2, *A*; see Fig E11 in this article's Online Repository at www.jacionline.org). This suggests a restoration of Treg-cell function, possibly through the wild-type allele of *CTLA-4*, as FOXP3 is a Treg-cell function regulator.[8] Unlike the previous reports with patients having haploinsufficiency of CTLA-4 expression, our patient showed even higher CTLA-4 expression in Treg cells (CD4$^+$CD25$^+$FOXP3$^+$) than did healthy donors (Fig 2, *B*).[1,2] Remarkably, the drug treatment effectively reduced CTLA-4 expression in Treg cells, as demonstrated by fluorescence-activated cell sorting and quantitative RT-PCR analyses (Fig 2, *B* and *C*), indicating a reduced proliferation of Treg cells as a proliferating Treg-cell preferentially expresses CTLA-4.[9] The drug treatment increased *TGF-β1* expression level, demonstrating that the drug successfully restores Treg-cell functionality (Fig 2, *C*; see Table E5).[10] The drug treatment restored CD3$^+$ T-cell level and attenuated inflammatory cytokine production such as IL-10, IL-17A, and IL-6 from patient-derived PBMCs (Fig 2, *D*; see Table E4 in this article's Online Repository at www.jacionline.org). Initial serum levels of IL-10, IL-17A, and IL-6 were higher in the patient than in her healthy parents, and the increased serum cytokine levels were reduced in a similar fashion by the drug treatment (see Fig E12 in this article's Online Repository at www.jacionline.org).

Clinically, treatment with abatacept without other immunosuppressants reduced diarrhea output by about 67% (from 3 L/d to 1 L/d) and her stool consistency has recently improved from watery to partially formed status. Remarkably, the blood transfusion volume has dropped to zero (Fig 2, *E* and *F*), allowing the patient to be free of the other immunosuppressants. She was discharged to outpatient care with partial parenteral nutrition after 3 years of hospitalization. Her direct Coombs test results turned negative after 8 months of abatacept treatment. After cessation of the steroid, hyperglycemia and adrenal insufficiency were easily controlled.

Here we report a direct effect of CTLA-4 dysfunction in human; enhancing the CTLA-4–mediated signal with CTLA-4-Ig toned down immune responses in a patient with severe autoimmune features, eventually achieving substantial clinical improvements. Therefore, it would be worthwhile to investigate patients with idiopathic autoimmune features for additional CTLA-4 mutations that may affect proper protein function and use CTLA-4-Ig as a potential therapeutic solution.

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Sangmoon Lee, MD[a]*
Jin Soo Moon, MD, PhD[b]*
Cho-Rong Lee, MS[c]*
Hye-Eun Kim, PhD[c]
Sun-Mi Baek, BS[c]
Solha Hwang, MS[b]
Gyeong Hoon Kang, MD, PhD[d]
Jeong Kee Seo, MD, PhD[b]
Choong Ho Shin, MD, PhD[b]
Hyoung Jin Kang, MD, PhD[b,e]
Jae Sung Ko, MD, PhD[b]
Sung Gyoo Park, PhD[c]
Murim Choi, PhD[a,b]

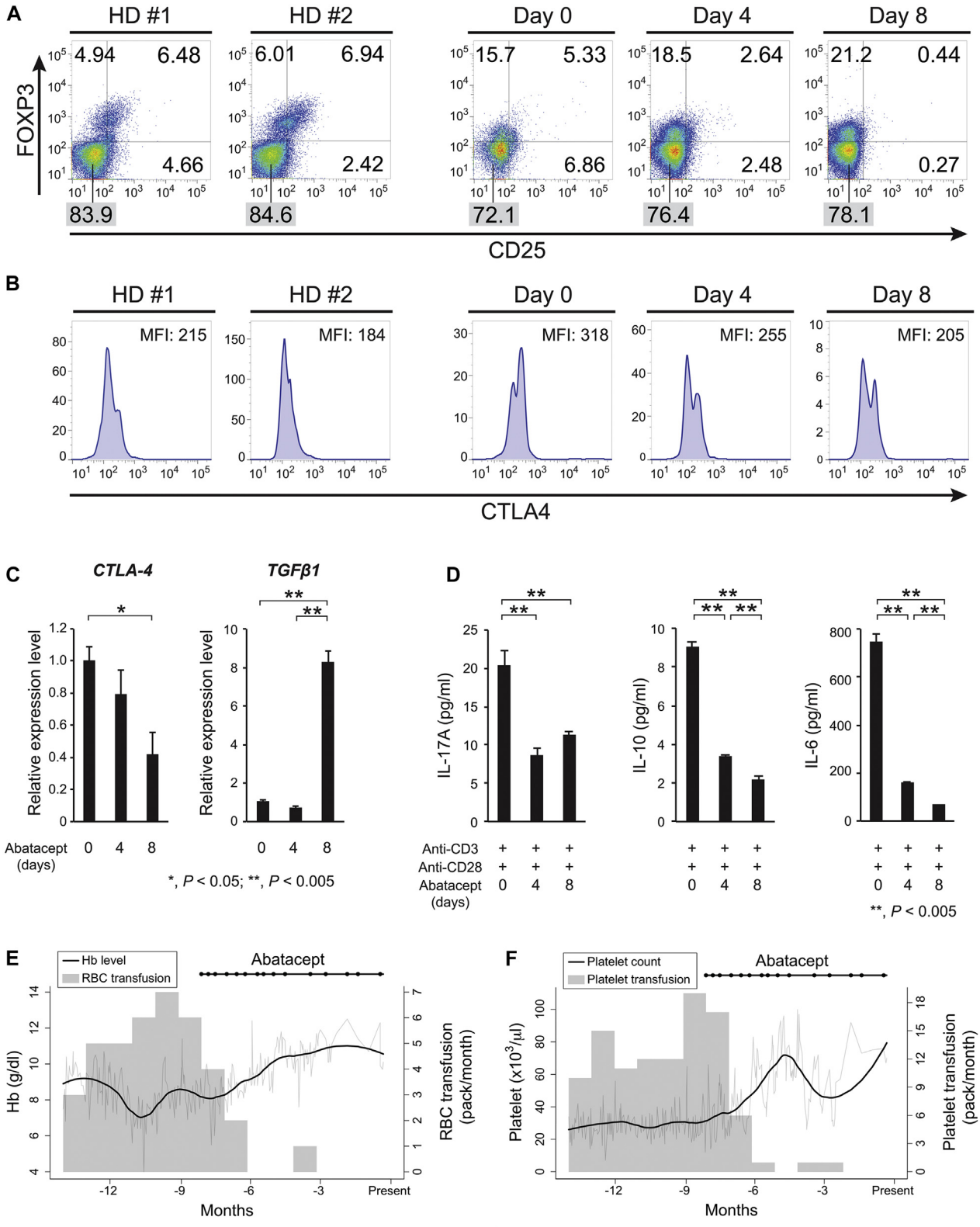

FIG 2. CTLA-4-Ig agent abatacept controls immune reactivity of the patient. **A**, Treg-cell ($CD4^+CD25^+FOXP3^+$) population in healthy donors (HD) and the patient. **B**, CTLA-4 expression level in $CD4^+CD25^+FOXP3^+$ cells. **C,** Quantitative RT-PCR of Treg-cell markers from the patient-isolated Treg cells. **D,** Concentration of cytokines from patient-derived PBMC culture supernatant. **E** and **F,** Patient blood levels of hemoglobin and platelet *(gray and smoothened black lines)* plotted against RBC and platelet transfusion rates *(gray bars)*. Period and actual administration of abatacept treatment are depicted as a black line with dots. *MFI*, Mean fluorescence intensity. $*P < .05$ and $**P < .005$.

From [a]the Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea; [b]the Department of Pediatrics, Seoul National University Hospital, Seoul, Korea; [c]the School of Life Sciences and Integrative Aging Research Center, Gwangju Institute of Science and Technology, Gwangju, Korea; [d]the Department of Pathology, Seoul National University Hospital, Seoul, Korea; and [e]the Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea. E-mail: kojs@snu.ac.kr. Or: sgpark@gist.ac.kr. Or: murimchoi@snu.ac.kr.

*These authors contributed equally to this work.

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