



## Letter to the Editor

## A de novo monoallelic CTLA-4 deletion causing pediatric onset CVID with recurrent autoimmune cytopenias and severe enteropathy



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## To the editor

Monoallelic mutations in *CTLA4* have been recently described in a small number of individuals with a phenotype of common variable immunodeficiency (CVID). The clinical features are variable and include hypogammaglobulinemia, autoimmune cytopenias, lymphoproliferative disease, and autoimmune enteropathy [1,2]. More recently, a large cohort study of 133 patients with CTLA-4 haploinsufficiency highlighted the susceptibility of these patients to infections with cytomegalovirus and Epstein Barr virus [3]. The majority of CTLA4 haploinsufficiency is caused by nonsense, missense, or frameshift mutations [3]. Only two patients diagnosed with CVID in adulthood have been reported to carry a large mono-allelic heterozygous deletion spanning the 2q33.2-2q33.3 region, which includes *CTLA4*, *CD28* and *ICOS* [3]. We now report a female patient with a de novo monoallelic deletion in *CTLA4* presenting with pediatric-onset CVID complicated with severe enteropathy and recurrent autoimmune cytopenias.

The proband was born to non-consanguineous parents of Italian descent with no family history of immunodeficiency or immune dysregulation. The proband presented with recurrent respiratory infections from the age of two years. At three and seven years of age, she presented with immune thrombocytopenic purpura (ITP) that responded well to treatment with high-dose intravenous immunoglobulin (IVIG). At eight years of age, the patient presented with her first episode of Coombs positive autoimmune hemolytic anemia (AIHA). Immunological evaluation at this time revealed pan-hypogammaglobulinemia (IgG 449 mg/dl, normal 633–1016; IgA: < 5 mg/dl, normal 41–315; IgM: 42 mg/dl, normal 56–261) leading to a diagnosis of CVID. The patient was started on IVIG replacement therapy and on prophylaxis with amoxicillin and cotrimoxazole. The patient had no evidence of infections with CMV, EBV or hepatitis. Due to refractory AIHA and ITP, the patient also received steroids and anti-CD20 treatment. At 14 years of age, immunological evaluation showed reduction of naïve T cells and recent thymic emigrants, expansion of CD4<sup>+</sup> and CD8<sup>+</sup> effector memory T cells and B cell maturational arrest at the IgM<sup>+</sup> memory stage (Supplemental Table 1). At this time, she developed chronic diarrhea. Histological examination revealed increased T cell infiltrate in the duodenum (Fig. 1A and B) and increased glandular

apoptotic bodies with an inflammatory cell infiltrate in the colonic lamina propria (Fig. 1C and D). Treatment with oral budesonide resulted in partial improvement. TNF inhibition with infliximab was initiated, but was stopped due to development of transaminitis. The patient was then put on adalimumab and azathioprine with partial clinical response. At 15 years of age, lung CT scan showed nodular lesions with ground glass abnormalities and opacification (Fig. 1E). The patient is currently 24 years old with a height and weight below the third percentile.

Targeted next generation sequencing (NGS) of the proband for 264 genes associated with primary immunodeficiency revealed a monoallelic deletion of exons 1–4 of *CTLA4*, which was not found in either parent (Fig. 1F). CGH array (180 K Agilent Technologies) confirmed the monoallelic deletion of 2q33.2-2q33.3 previously reported in two patients with adult-onset CVID, which also includes *CTLA4*, *ICOS*, and *CD28* [3]. Flow cytometric evaluation of CTLA-4 expression showed severely decreased protein expression in activated CD4<sup>+</sup> T cells and an almost complete lack of Foxp3<sup>+</sup> T regulatory cells (Tregs) (Fig. 1G). (CTLA-4 MFI: HD 16,5; Pt:9,77) (percentages of CTLA-4 + Foxp3 + CD4<sup>+</sup> T cells (%) HD: 3,6; Pt: 0,18). One of the previously reported patients had arthritis, viral, and fungal infections, while the other developed AIHA and CVID [3]. In contrast, our proband presented with very early-onset respiratory infections at the age of two years, followed by the development of autoimmune cytopenias and hypogammaglobulinemia in childhood. Environmental exposures, modifier genes, and/or epigenetic modifications may be responsible for the differences observed in these patients. Of note, the index patient developed severe B cell lymphopenia over time (Supplemental Table 1), as previously described in a subset of CTLA-4 haploinsufficient patients [3].

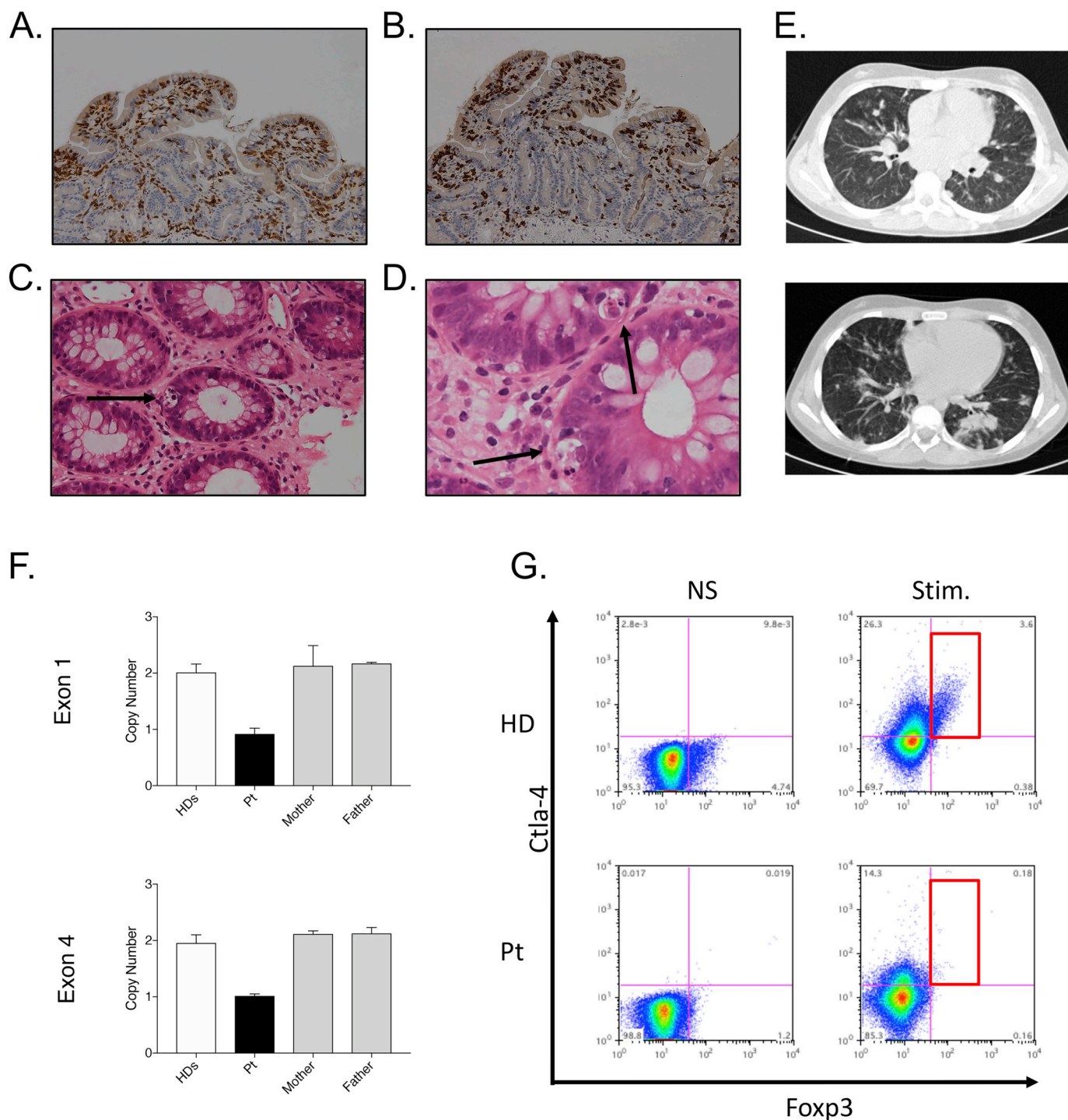
Here, we report a pediatric-onset CVID patient harbouring a de novo 2q33.2-2q33.3 monoallelic deletion leading to nearly absent CTLA4 expression. This is the third patient worldwide harbouring this deletion, and the second one with a de novo deletion. The patient's phenotype resulted particularly complex and resembles the previously described presentation in CTLA-4 haploinsufficient patients [3], although in a more severe form and with an early-onset. Considering the complexity of this disorder, further longitudinal studies are warranted in order to better define the appropriate therapeutic approach for affected patients.

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**Fig. 1.** Clinical, immunological and genetic findings of the index patient. A and B. increased number of intraepithelial T lymphocytes, CD3 and CD8 immunohistochemical staining (10×). C and D. Large bowel mucosa reveals increased glandular apoptotic bodies (arrows) and a mild inflammatory cell infiltration in the lamina propria (HE, 20× e 40×) E. Lung CT scan of the index patient showing nodular lesions and ground glass abnormalities. F. Copy number variation (CNV) for CTLA-4 for exons 1 and 4 (upper and lower panel respectively) for the index patient (Pt), healthy parents (Mother and Father), and three healthy controls (HDs). G. Flow cytometric evaluation of CTLA-4 expression on CD4+ T cells at resting state (NS) and upon anti-CD3 stimulation (Stim) from the index patient (Pt) and an age-matched healthy control (HD).

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clim.2018.10.007>.

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## Conflict of interest

The authors declare no conflict of interest.

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