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RAPID COMMUNICATION

An interleukin 10 Receptor alpha (IL-10RA) variant with a compound heterozygote mutation shows normal IL-10 signaling activity

Loss-of-function mutations of interleukin 10 receptor alpha subunit (*IL*-10RA) (homozygotes or compound heterozygotes) are associated with pediatric colitis and diarrhea. These mutations always drive a significant increase in serum interleukin-10 (IL-10) levels, while such high levels of IL-10 fail to suppress intestinal inflammation, at least in part by failure to suppress the release of some cytokines such as interleukin 1 beta (IL-1β) and tumor necrosis factor-alpha (TNFα).[1,2] However, at present, whether a novel missense variant has a pathogenic effect is mainly determined according to the guidelines issued by the American College of Medical Genetics and Genomics (ACMG). Without functional verification, a variant of uncertain significance (VUS) will be easily made, or a variant will be misjudged as pathogenic or benign. Thus, a function test of a novel mutated *IL*-10RA is needed to determine whether colitis was attributed to such a mutation.

Here we reported a 14-month-old boy who had diarrhea 5-to-6 times a day soon after birth. Gross blood and mucus were frequently seen in his loose stools. Meantime, he had eczema in the body. He was diagnosed as food-protein induced allergic proctocolitis (FPIAP) because the symptoms were alleviated after amino acid infant formula was introduced. However, frequent diarrhea, in combination with rectal bleeding and increased streaks of mucus in the stools, may worsen after adding complementary food and vaccination even if he was one year old. He was referred to our clinic for a colonoscopy. Severe colonic lymphonodular hyperplasia (LNH) with mucosal erosion in colonoscopy (Fig. 1A, I) led to a diagnosis of chronic colitis with LNH. He was advised to be fed with amino acid formula and treated with mesalazine and achieved sustained clinical remission.

After the patient was discharged, he and his parents received whole exome sequencing in another hospital. It was shown that he has a compound heterozygote mutation of *IL*-10RA (chr11:117860270, NM_001558 c.302(exon3)G > A, p.R101Q, from mother; chr11:117864125, NM_001558 c.537(exon4)G > A, p.T179T, from father) (Fig. 1B, C). Mutation of c.537G > A is a pathogenic mutation by disrupting ribonucleic acid (RNA) splicing and is a hotspot mutation in the Chinese Han population.[3] Mutation of c.302G > A is a point mutation in exon 3 of the *IL*-10RA gene, resulting in the replacement of an arginine residue at position 101 with glutamine. In the previous clinical studies, the variation of p.R101Q was considered likely pathogenic in the absence of functional study.[3,4] However, according to ACMG, p.R101Q should be judged as a VUS (PM2 + PM5 + PP3). Our patient lacked typical symptoms, endoscopic characteristics, and imaging features induced by *IL*-10RA mutation, so a diagnosis of IL-10RA associated immunodeficiency or very early onset-inflammatory bowel disease (VEO-IBD) was not made. Instead, the patient's symptoms were considered to be attributed to FPIAP, and he was advised to feed continuously with amino acid formula and receive treatment with mesalazine. A half-year after persistent alleviation of symptoms, the patient chose to have a regular diet and stopped mesalazine but persisted in avoiding milk and egg.

The patient exhibited continued remission when he was three years old with a regular diet and without medication. Thus, he was admitted to our hospital for a follow-up colonoscopy examination and to receive an evaluation of IL-10R function. Colonoscopy displayed normal intestinal mucosa with mild LNH (Fig. 1A, II). Histopathological analysis of his biopsy of sigmoid colon mucosa during the first colonoscopy revealed some atypical glands, prominent lymphocyte infiltration, and lymphoid follicles in mucosa

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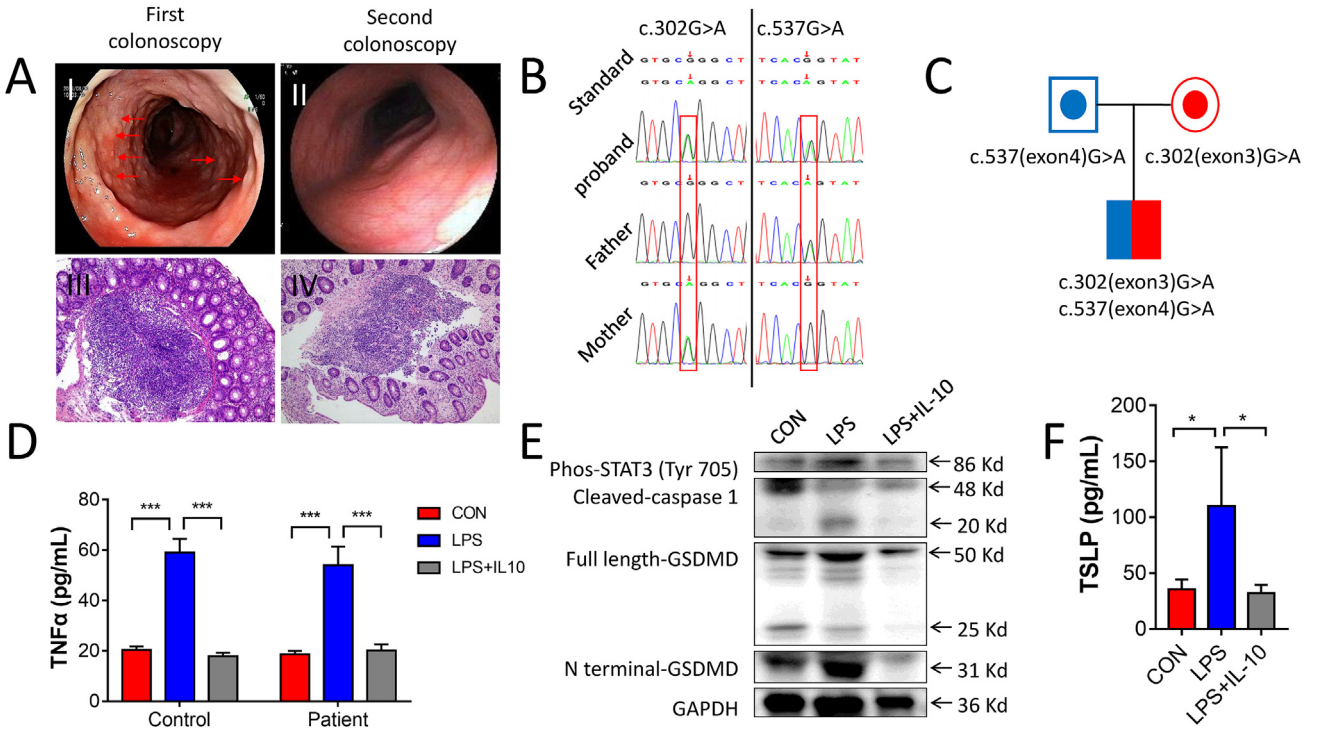

Figure 1 Genetic and clinical characteristics of gene mutation and functional verification. **(A)** Colonoscopy results and histopathological analysis of the patient. I. The first colonoscopic findings showed severe colonic lymphonodular hyperplasia (LNH) with mucosal erosion. II. The second colonoscopic findings displayed normal intestinal mucosa with mild LNH. III. The histopathological analysis of sigmoid colon tissue during his first colonoscopy revealed some atypical glands, obvious lymphocytes infiltration, and lymphoid follicle in mucosa lamina propria of sigmoid colon tissue (hematoxylin and eosin staining, $\times$100). IV. The histopathological analysis of the sigmoid colon tissue in his follow-up colonoscopy showed a few focal lymphoid follicles, a mild decrease in goblet cells of the glands, and a slight increase in lymphocyte infiltration, without granuloma and erosion (hematoxylin and eosin staining, $\times$100). Red arrows indicate raised lymphoid follicular hyperplasia. **(B)** Sequencing electropherograms of genomic DNA from the index patient and unaffected parents, showing the c.302(exon3)G $>$ A (p.Arg101G1n) and c.537(exon4)G $>$ A (p.Thr179Thr) mutation. **(C)** Pedigree of the family involved in this study. **(D)** PBMCs from the control subject and the patient were stimulated with PBS as control, LPS, and LPS + IL-10. TNFα production in the supernatant was measured by ELISA 24 h after stimulation (n = 5). **(E)** Lysates of PBMCs from the patient were used to detect phosphorylation of STAT3 and expression of cleaved caspase-1, full-length GSDMD, and N-terminal GSDMD by Western blot. GAPDH was visualized as the loading control. **(F)** The supernatant of PBMCs from the patient was also used to determine TSLP production (n = 5). Statistically significant differences were analyzed using a one-sample t-test. *$P < 0.05$; ***$P < 0.001$. Data were presented as mean $\pm$ standard deviation.

lamina propria (Fig. 1A, III). In addition, a biopsy of the mucosa in the sigmoid colon showed a significant reduction in inflammation during his follow-up. It showed a few focal lymphoid follicles, a mild decrease in goblet cells of the glands, and a slight increase in lymphocyte infiltration, without granuloma and erosion (Fig. 1A, IV). These findings further ruled out the possibility of VEO-IBD. Laboratory tests revealed a slight increase in serum IL-10 (10 pg/mL, normal range: 0—9.1 pg/mL), which is less than the cutoff value (>33.05 pg/mL, sensitivity: 1.0, specificity: 0.84) suggested for the presence of disease-causing mutation of *IL*-10RA in VEO-IBD according to our previous research.[5] Therefore, we have analyzed whether the IL-10R biological activity deteriorated by the compound heterozygous variants IL-10RA c.302G $>$ A/c.537G $>$ A. To verify this, peripheral blood mononuclear cells (PBMCs) were isolated and stimulated with lipopolysaccharide (LPS) with or without recombinant human IL-10. Compared to phosphate buffered saline (PBS), LPS increased TNFα production while IL-10 inhibited LPS-induced TNFα production (Fig. 1D), reflecting an unimpaired IL-10RA-mediated anti-inflammation function in this patient. We next determined the phosphorylation of signal transducer and activator of transcription 3 (STAT3) as a marker of T helper cell 17 (Th17) immune response upon LPS stimulation with or without IL-10. Compared to PBS, LPS stimulation significantly increased STAT3 phosphorylation at Tyr 705, while IL-10 reversed this increase, indicating suppression of Th17 by IL-10 (Fig. 1E). Careful observation found that this patient's serum level of cytokine IL-1β was within the normal range. IL-1β is a potent inflammatory cytokine released upon cell pyroptosis and contributes to colitis via IL-10R signaling.[1] To assess the suppressive role of IL-10R in pyroptosis, PBMCs were stimulated with LPS with or without IL-10. LPS stimulation triggered pyroptosis with increased N-terminal Gasdermin D (GSDMD). This pyroptosis was as

least in part mediated via caspase-1 as LPS led to the increased level of cleaved caspase-1 (Fig. 1E). However, adding IL-10 remarkably reversed these phenomena (Fig. 1E), suggesting an IL-10R-mediated suppression of pyroptosis. These results indicate the existence of an IL-10R-related anti-inflammatory role in this patient, coinciding with a nearly normal range of TNFα (12.5 pg/mL, normal range: 0—8.1 pg/mL) and a normal range of IL-1β ($<$5 pg/mL, normal range: 0—5 pg/mL) in the serum and also the normal colonoscopy manifestation after he eliminated cow milk and egg from his diet, without using any glucocorticoid, immunosuppressive agent, or biological agents. In fact, if a patient's colitis is caused by *IL*-10RA mutation, the patient is usually resistant to multiple drug treatments and requires hematopoietic stem cell transplantation to relieve the disease. Our patient did not have these characteristics. In addition, we found that PBMCs from our patient retained the ability of IL-10R-mediated suppression in LPS-induced thymic stromal lymphopoietin (TSLP) production (Fig. 1F), which has acted as a master of intestine inflammation.

By searching the literature, we found that previously reported patients with c.302G $>$ A (p.R101Q) exhibited mild clinical symptoms and only presented as rectal ulcers, followed by continuous remission. All these findings are obviously different from the typical characteristics of patients with previously reported *IL*-10RA mutations which have 100% penetrance, resistance to multiple medications, and need for hemopoietic stem cell transplantation and protective ileostomy to reach disease remission. Combined with the reported clinical features of our patient and the normal function of *IL*-10RA suggested by *in vitro* functional tests, we believed that this mutation in *IL*-10RA (chr11:117860270, NM_001558 c.302G $>$ A, p.R101Q) could be assessed as a likely benign mutation according to the ACMG guidelines after adding the evidence of BS3.

Author contributions

J.J.L. and X.Y.C. performed the experiments, analyzed the results, and wrote the manuscript. Y.X. coordinated the study and wrote the manuscript. All authors approved the final version of the manuscript.

Data availability

All data generated and analyzed in this study are included in this published article.

Conflict of interests

All authors have no competing interests to declare.

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