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SHORT REPORT

Novel de novo mutations of the interleukin-10 receptor gene lead to infantile onset inflammatory bowel disease

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Abstract

Background and aims: Defects in the interleukin 10 (IL-10) signalling pathway have been shown to cause very early onset inflammatory bowel disease (IBD). We report a patient with severe infantile-onset IBD with a compound heterozygous IL-10 receptor alpha subunit (IL-10RA) mutation, one of which was paternally-inherited and the other occurring *de novo*. Methods:

Abbreviations: GWAS, genome-wide association studies; HSCT, haematopoietic stem cell transplantation; IL-10RA, IL-10 receptor alpha subunit; IL-10RB, IL-10 receptor beta subunit; IBD, inflammatory bowel disease; IL-10, interleukin 10; LPS, lipopolysaccharide; NBT, nitro blue tetrazolium; PCDAI, Paediatric Crohn's Disease Activity Index; PBMC, peripheral blood mononuclear cells; pSTAT3, phosphorylated STAT3; Treg, regulatory T-cells; STAT3, signal transducer and activator of transcription 3; WBC-Tc99m, technetium-tagged white blood cells scan; TPN, total parental nutrition; TNFα, tumour necrosis factor alpha.

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Deep sequencing of IL-10, IL-10RA and IL-10 receptor beta subunit (IL-10RB) were performed. Peripheral blood mononuclear cell (PBMC) surface expression of IL-10RA was analysed by flow cytometry. IL-10 signalling pathway was examined by measuring phosphorylated STAT3 in PBMC cultured in the presence of IL-6 or IL-10. Result: We identified a missense mutation in exon 4 of IL-10RA (c.583T>C) in one allele and a nonsense mutation in exon 7 of IL-10RA (c.1368G>T) in the other allele. Neither mutation has been reported previously. The patient has functional IL-10RA deficiency despite normal IL-10RA expression. Conclusion: This represents the first case report of a *de novo* mutation of IL-10RA that is associated with very early onset severe IBD. Therefore, IL-10 pathway defect should be considered in patients with infantile-onset IBD even if the parents are non-consanguineous.

1. Case report

The patient was born at 35 weeks gestation with a birth weight of 2.5 kg (50th percentile) to non-consanguineous white Caucasian parents. He was breast fed and remained healthy during the first month of life. Following the introduction of a cow's milk formula feeds he developed diarrhoea and eczema. These symptoms were attributed to cow's milk protein allergy and the formula was changed to a hypoallergenic amino acid based feed. There was no improvement and at four months the patient was referred to a tertiary centre for investigation of failure to thrive and bloody diarrhoea. Clinical examination revealed anal fissures and ulcers. Endoscopy showed gastritis, duodenitis and florid colitis of the rectum and sigmoid colon; complete colonoscopy was abandoned due to friable mucosa. Immunological investigations including nitroblue tetrazolium (NBT), immunoglobulins, and lymphocyte subsets were normal, but there was evidence of chronic systemic inflammation with raised erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), thrombocytosis and hypoalbuminaemia.

The patient was initially treated with corticosteroid, azathioprine, gut rest and total parental nutrition (TPN) with minimal improvement. He also had multiple courses of antibiotics (ciprofloxacin and metronidazole) for perianal fissures and fistulae, with some symptomatic improvement. An induction course of infliximab (5 mg/kg at week 0, 2, 6) at 8 months of age resulted in a partial response enabling reintroduction of enteral feeding. However, his disease subsequently flared and was refractory to further doses of infliximab.

At 2 years of age, he had a further flare of his colitis with extensive fissuring and destructive fistulising perianal disease. This was further complicated by multiple episodes of severe Gram-negative sepsis. He was treated with systemic antibiotics, gut rest, TPN and finally a colectomy with formation of an end-ileostomy. His perianal disease markedly improved thereafter, but he continued to have frequent abdominal pain.

Currently at 10 years of age, he has significant growth failure (height Z score −3.05; weight Z score −3.91). He continues to be symptomatic and has elevated systemic inflammatory markers. A recent Paediatric Crohn's Disease Activity Index (PCDAI) was 45, indicating ongoing severe disease. A repeat gastroscopy shows microscopic oesophagitis, gastritis and duodenitis, without granulomata. He suffers from severe food aversion and receives his entire nutritional requirements in the form of an elemental formula via a gastrostomy. Besides mild eczema, the patient had pyoderma gangrenosum but not folliculitis. He does not have any other extra-intestinal manifestations of IBD or autoimmune disease.

2. Identification of novel IL-10RA mutation

To investigate a possible IL-10 signalling pathway defect causing early onset IBD,[1–3] molecular genetic testing was performed as previously published.[4] In brief, deep sequencing of IL-10, IL-10 receptor alpha subunit (IL-10RA) and IL-10 receptor beta subunit (IL-10RB) was performed. We identified a missense mutation in exon 4 of IL-10RA (c.583T>C) in one allele, leading to replacement of isoleucine by threonine at position 169 (Ile169Thr); and a nonsense mutation in exon 7 of IL-10RA (c.1368G>T) in one allele, leading to replacement of glutamic acid with a premature stop codon (Glu431X). (Fig. 1).

Both parents were healthy. The father of the proband was heterozygous for the mutation in exon 4 of IL-10RA (c.583T>C), whilst the mother of the proband did not carry any of the above mentioned mutations, although we could not rule out a germline mutation. This indicates that the proband inherited the mutation on exon 4 from his father and likely developed a novel de novo mutation in exon 7. The Ile169Thr mutation is located on the 'C' beta-strand within the D2 region of the extracellular domain of IL-10RA.[5] It is oriented inwardly within the beta-sandwich fold. Whilst it is not proximal to the IL-10/IL-10RA interface, the isoleucine to threonine mutation would introduce a polar side chain into the hydrophobic core of the beta-sandwich macrostructure that could disrupt it or prevent proper folding. The missense mutation Glu431X is in the intracellular portion and therefore we would expect that even with the truncation, there would still be an expressed product. However, the truncation would remove Tyr residues Y446 and Y496 that are important for mediating STAT3 binding.

3. IL-10 signalling pathway defect

Given the patient's mutations are novel, it was important to demonstrate a functional defect in IL-10 signalling. Peripheral blood mononuclear cells (PBMC) were isolated via Ficoll-Paque centrifugation. Surface expression of IL-10RA was analysed by flow cytometry, which demonstrated comparable IL-10RA expression between the patient and the control (Fig. 2A). After IL-10 binds to its receptor, the principal signalling

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