

Genetic Diagnosis Guides Treatment of Autoimmune Enteropathy



Autoimmune enteropathy (AIE) is a severe form of enteropathy characterized by chronic diarrhea refractory to any exclusion diet and associated with autoimmunity.^{1,2} Despite this clear definition, diagnosis is challenging. The presence of anti-enterocyte antibodies against the AIE-75kDa antigen³ is a strong but inconstant biomarker. In a recent cohort of 40 AIE patients, anti-enterocyte antibodies were reported in only 14% (4/28) of the cases, likely caused by the high frequency of patients with primary hypogammaglobulinemia.² Moreover patients may display celiac anti-transglutaminase antibodies.⁴ The common histopathologic presentation of AIE includes intestinal villous atrophy with variable lymphocytic infiltration and various features of follicular lymphoid hyperplasia, cryptitis, graft-versus-host disease-like lesions, and loss of Paneth and goblet cells.^{2,4} This mixed histopathologic pattern combined with inefficacy of gluten-free diet allow differentiating AIE from celiac disease.^{1,2,4} Treatment remains challenging because AIE patients are only variably improved by steroids and immunomodulators.⁴ Therefore, identification of the underlying molecular mechanism is crucial to treat unresponsive patients. Genetic studies have revealed that several monogenic inborn errors of immunity, notably *NFKB1* (nuclear factor kappa B subunit 1), *CTLA4* (cytotoxic T-lymphocyte-associated protein 4) haploinsufficiency, and *LRBA* (LPS responsive beige-like anchor protein) deficiency may manifest by AIE only at adulthood (reviewed in⁵). The latter finding prompted us to screen a cohort of 48 adult patients with AIE by next-generation sequencing (Supplementary Methods). Representative endoscopic findings and histologic features are shown in Figure 1A and B. Clinical features are summarized in Figure 1C. Malnutrition (body mass index <19), anemia, and primary immunoglobulin deficiency were present in 67% of cases. Small bowel inflammation consisted mainly in villous atrophy in 30/48 (63%), and intraepithelial and/or lamina propria lymphocytosis in 39/48 patients (81%). Intestinal lymphocytes had a normal phenotype and TCR γ rearrangements showed a polyclonal profile in intestinal biopsies except in 2 patients who developed intestinal CD4⁺ T-cell lymphoproliferations. Chronic gastritis and colitis were present in 39/48 patients (81%). Eight patients with primary hypogammaglobulinemia and abnormal hepatic tests and/or liver imaging had biopsy-proven nodular regenerative hyperplasia. Main peripheral blood abnormalities were lymphopenia (34/48), particularly B lymphopenia, in keeping with the high frequency of primary hypogammaglobulinemia.

Pathogenic variants were identified in 20/48 patients (41.6%) (Supplementary Table 1). In 9 cases, the pathogenic variants were previously reported as disease causing. Five other cases displayed nonsense variants that were considered pathogenic according to American College of Medical Genetics and Genomics guidelines.⁶ Functional validation was performed for missense variants that had not been previously described in the literature (Supplementary Figure 1).

Before diagnosis, gluten-free diet (20/30; 67%) and, in patients with primary hypogammaglobulinemia, immunoglobulin replacement therapy (19/32; 59%) were ineffective in all cases. Steroids (34 patients; 71%), frequently budesonide (27/48), induced partial clinical remission and partial mucosal recovery in all except 2. Thirteen patients (27%) received immunomodulators and 26 (54%) at least 1 biotherapy. Anti-tumor necrosis factor- α agents (19 patients; 40%) induced partial and/or transient clinical remission without any histologic effect in all except 2. In 8 patients, molecular diagnosis enabled targeted therapy. Abatacept, a soluble fusion protein analogue to CTLA-4, induced durable ($n = 3$) or transient ($n = 3$) remission with relapse occurring after 2–3 years. In 1 patient with *STAT3* gain of function (GOF) variant, JAK inhibitor ruxolitinib induced complete clinical and histologic remission still persistent after 4 years.⁷ In contrast, ruxolitinib had no effect in 1 patient carrying a *STAT1* GOF variant.

Patients with identified pathogenic gene variants had earlier onset of enteropathy ($P = .0015$) (Figure 1D). Malignant gastrointestinal disorders were observed in 9 patients and developed more rapidly in those with identified genetic variants ($P = .0021$) (Figure 1D). Nevertheless, identification of a pathogenic variant did not significantly affect global survival. Four patients with (*CTLA4*, $n = 2$) or without ($n = 2$) identified pathogenic variants developed gastric dysplasia ($n = 2$) or gastric adenocarcinoma ($n = 2$). All 4 had atrophic gastritis, *Helicobacter pylori* being only detected in 1 patient before onset of gastric adenocarcinoma. Endoscopic mucosal resection was performed in all cases of gastric dysplasia, and total gastrectomy with radiotherapy in 1 patient with gastric cancer. Duodenal adenoma developed in 1 patient with *STAT3* GOF variant and villous atrophy and was

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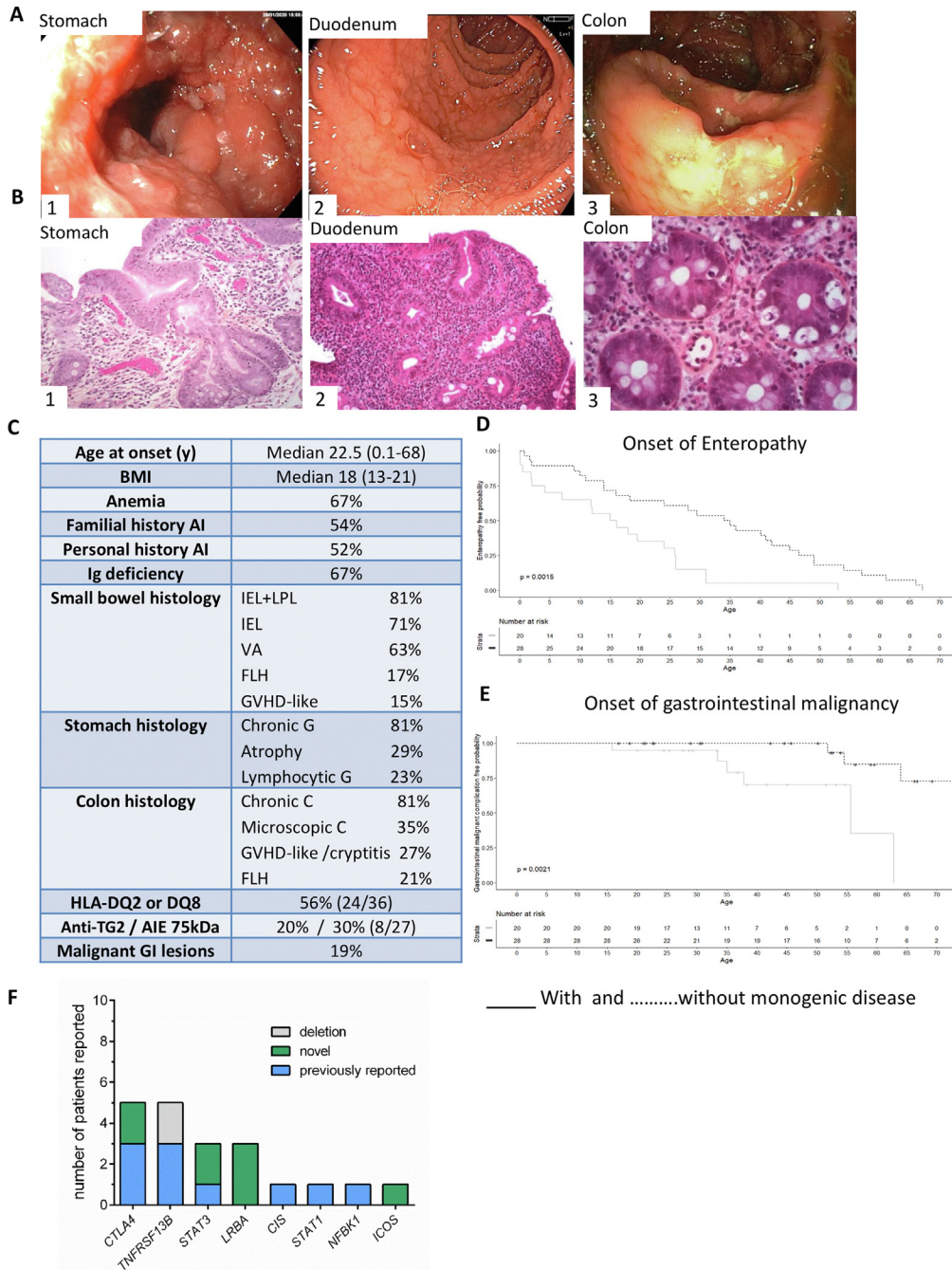


Figure 1. (A) Representative endoscopic aspects in patients with *CTLA4* variants and AIE. (1) Ulcerative gastritis with pseudo-obstruction in patient P9. (2) Duodenal villous atrophy in P1. (3) Colitis with ulcerations in P9. (B) Representative histologic lesions in patients with AIE. (1) Chronic fundic atrophic gastritis with intestinal metaplasia and focal low-grade dysplasia ($\times 200$) in P9 carrying a *CTLA4* variant. (2) Chronic duodenitis in P1 with *CTLA4* mutation with severe villous blunting and increased IEL numbers ($\times 200$). (3) Colitis with GVHD-like lesions and cryptic apoptotic bodies ($\times 400$) in a patient without identified pathogenic variant (description of numbered patients is provided in [Supplementary Table 1](#)). (C) Clinical features of the 48 patients with AIE. (D) Kaplan-Meier enteropathy-free survival curve of patients with (solid line) or without molecular diagnosis (dashed line). (E) Kaplan-Meier gastrointestinal malignant-free survival curve of patients with (solid line) or without molecular diagnosis (dashed line). (F) Number of patients for each individual molecular diagnosis. anti-TG2, antitransglutaminase of type 2; AI, autoimmune; AIE-75kDa, autoimmune enteropathy-related 75-kilodalton antibody; BMI, body mass index; C, colitis; IEL, intestinal intraepithelial hyperlymphocytosis; FLH, follicular lymphoid hyperplasia; G, gastritis; GI, gastrointestinal; GVHD, graft-versus-host disease; LPL, lamina propria hyperlymphocytosis; VA, villous atrophy.

endoscopically resected. Colonic dysplastic adenomas occurred in 3 patients (*CTLA4*, n = 1; *NFKB1*, n = 1; no diagnosis, n = 1), all treated by endoscopic resection. Among them, high-grade dysplasia was observed in the

mutated *NFKB1* patient. Low-grade intestinal CD4⁺ T-cell lymphoproliferation was observed in 2 patients: 1 patient without identified variant but treated by azathioprine still displayed conspicuous CD4⁺ T-cell intraepithelial

infiltration without detectable clonal TCR γ rearrangement 2 years after azathioprine discontinuation. The second patient with TNFRSF13B variant progressed toward fatal high-grade CD4⁺ T-cell lymphoma. Extraintestinal tumors were diagnosed in 2 other patients without identified pathogenic variants: 1 thymoma (4 years after first detection of hypogammaglobulinemia, a posteriori leading to diagnosis of Good syndrome) and 1 papillary thyroid cancer treated by surgery. Six patients died and 5 patients were lost to follow-up.

In summary, 40% of our adult AIE patients carried pathogenic variants providing clues for diagnosis, treatment, and follow-up. Diagnosis of AIE can be difficult and notably mistaken for refractory celiac disease. Because 30%–50% of adult AIE can display anti-transglutaminase antibodies^{4,8} identification of genetic variant allows diagnosis of AIE. Tailor-made therapeutics were available for more than half of the patients who received a molecular diagnosis (patients with variants in *CTLA4*, *LRBA*, *STAT3*, and *STAT1*; 12/20; 60%). Herein, 8 patients received such therapy. All patients responded except 1 patient with *STAT1* GOF and very severe disease. Along this line, a recent study reported significant improvement in 14/17 patients with *STAT1* or *STAT3* GOF variants treated with JAK-inhibitors.⁹ Finally, this study highlights the risk of malignant transformation associated with AIE and their earlier onset in patients with identified variants, in keeping with previous data demonstrating increased risk of gastric neoplasia in *CTLA4*-mutated patients.¹⁰ Overall these observations stress the need for early genetic diagnosis and regular endoscopic follow-up of these patients.

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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at <https://doi.org/10.1016/j.cgh.2022.07.030>.

References

1. Schieppatti A, et al. *Gut* 2022 Jun 8;gutjnl-2021-326645.
2. Villanacci V, et al. *Clin Immunol* 2019;207:10–17.
3. Kobayashi I, et al. *Gastroenterology* 1999;117:823–830.
4. Akram S, et al. *Clin Gastroenterol Hepatol* 2007;5:1282–1290; quiz 1245.
5. Charbit-Henrion F, et al. *Mucosal Immunol* 2021;14:1017–1037.
6. Richards S, et al. *Genet Med* 2015;17:405–424.
7. Parlato M, et al. *Gastroenterology* 2019;156:1206–1210.
8. Scialom S, et al. *PLoS One* 2015;10:e0125024.
9. Forbes LR, et al. *J Allergy Clin Immunol* 2018;142:1665–1669.
10. Schwab C, et al. *J Allergy Clin Immunol* 2018;142:1932–1946.

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Conflicts of interest

The authors disclose no conflicts.

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Supplementary Methods

Study Design and Primary Goal

Forty-eight adult patients with AIE were consecutively recruited for genetic analysis through multidisciplinary consultation meetings in 9 adult French university hospitals (AP-HP-Centre-Université Paris Cité [n = 32], AP-HP-Nord Université Paris Cité [n = 8], AP-HP-Université Paris Sorbonne [n = 2], CHRU de Besançon [n = 1], CHRU de Nantes [n = 1], CHU de St Etienne [n = 1], CHRU de Tours [n = 1], CHRU de Nîmes [n = 1], and CHRU de Lille [n = 1]).

Inclusion Criteria

All patients had chronic diarrhea and histopathologic features of AIE refractory to any diet.¹ Enteropathy induced by sartans, checkpoint inhibitors, and refractory celiac disease were excluded. Clinical and biologic data were assessed as described.²

Outcome

Date at onset of enteropathy and of gastrointestinal malignant complications were compared between patients with and without identified pathogenic variants. Response to treatment was assessed retrospectively and prospectively. Clinical remission was defined by 50% reduction in stool frequency and recovery of at least 50% of body weight loss. Histologic response was defined by total or partial mucosal healing. Follow-up extended from diagnosis of enteropathy to December 31, 2020.

Genetic Analysis

Genetic analysis was conducted from January 2015 to December 2020. Some patients were also investigated by other research laboratories (P9 and P19,³ P14,⁴ and P18⁵). We published 1 case (P3^{6,7}).

Targeted Next-Generation Sequencing and Whole Exome sequencing. Next-generation sequencing was performed on genomic DNA extracted from peripheral blood mononuclear cells, using custom-made targeted panels (TNGS) dedicated to intestinal disorders⁶ or using whole exome sequencing in trios (P3, P4, P5).⁷ Panel for TNGS described in⁶ has been updated with genes described in Charbit-Henrion et al⁸ and in Uhlig et al.⁹ Genomic DNA libraries were captured by hybridization using either Agilent Sure Select All Exon V5 or V6 (Agilent, Les Ulis, France) for whole exome sequencing or biotinylated complementary 120-pb RNA baits designed with SureSelect SureDesign software to cover all exons of the selected genes for TNGS. Data analysis was performed using the in-house platform POLYWEB created by the Bioinformatics core facilities of Université Paris Cité and Institut Imagine to prioritize rare and

potentially damaging variants according to the American College of Medical Genetics guidelines,¹⁰ OMIM identity, allele frequency in control populations (gnomAD), conservation (UCSC genome browser, 100 vertebrates), protein function and expression, and phenotypic assessment. Consequences of variants on protein function were predicted using Combined Annotation-Dependent Depletion score (<https://cadd.gs.washington.edu>) and Splice AI (<https://spliceailookup.broadinstitute.org>). All variants were confirmed by Sanger sequencing and familial segregation was analyzed when DNA from family members was available.

Functional Validation. Functional validation was performed in patients carrying missense variants not previously reported. In P4 with *STAT3* GOF mutation, transcription of Suppressor of Cytokine Signaling 3 (SOCS3) was studied in EBV transformed B cell lines as described.^{6,7} In P9, B-cell subsets were assessed by flow cytometry.³ In P18 and P19, LRBA expression was assessed by flow cytometry and Western blot.^{5,11} In P1, P7, and P20, CTLA4 expression was assessed by flow cytometry.^{3,11}

Statistical Analysis

Frequencies were compared using the Fisher exact test. Global survival, enteropathy-free survival, and gastrointestinal malignant/premalignant-free survival were estimated using Kaplan-Meier estimates and survival differences using the log-rank test. For each analysis, comparison was performed between patients with and without identified pathogenic variant. Statistical analyses used the R software version 4.0.2 (Vienna, Austria). $P < .05$ was considered statistically significant.

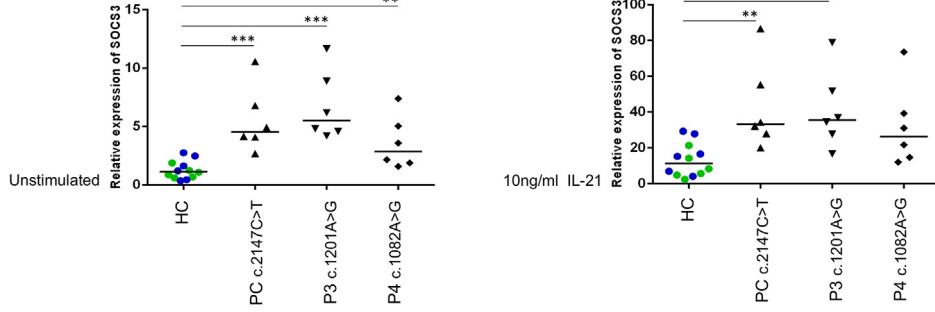
References

1. Villanacci V, et al. *Clin Immunol* 2019;207:10–17.
2. Scialom S, et al. *PLoS One* 2015;10:e0125024.
3. Fusaro M, et al. *J Allergy Clin Immunol* 2021;147:737–737.
4. Dragon-Durey MA, et al. *J Immunol* 2001;166:7612–7616.
5. Besnard C, et al. *Clin Immunol* 2018;188:52–57.
6. Charbit-Henrion F, et al. *J Crohns Colitis* 2018;12:1104–1112.
7. Parlato M, et al. *Gastroenterology* 2019;156:1206–1210.
8. Charbit-Henrion F, et al. *Mucosal Immunol* 2021;14:1017–1037.
9. Uhlig HH, et al. *J Pediatr Gastroenterol Nutr* 2021;72:456–473.
10. Richards S, et al. *Genet Med* 2015;17:405–424.
11. Mazerolles F, et al. *Front Immunol* 2018;9:718.

Supplementary References

1. Schubert D, et al. *Nat Med* 2014;20:1410–1416.
2. Tuijnenburg P, et al. *J Allergy Clin Immunol* 2018;142:1285–1296.
3. Fusaro M, et al. *J Allergy Clin Immunol* 2021;147:734–737.
4. Arts P, et al. *Genome Med* 2019;11:38.
5. Lee JJ, et al. *J Allergy Clin Immunol* 2010;126:1234–1241.
6. Dragon-Durey MA, et al. *J Immunol* 2001;166:7612–7616.
7. Uzel G, et al. *J Allergy Clin Immunol* 2013;131:1611–1623.

A STAT3 GOF



B CTLA4

	HC	P1	P7	P20
CTLA4 MFI in CD4 ⁺ CD25 ⁺ CD127 ^{low}	1549	816 (52%)	933 (60%)	826 (53%)
CTLA4 MFI in CD4 ⁺ CD25 ⁺ FOXP3 ⁺	2161	1058 (49%)	1172 (54%)	1079 (49%)

C NFKB1

	HC	P8
CD19 ⁺ CD27 ⁺ IgD ⁻	9-18%	3%
CD19 ⁺ CD21 ^{low} CD38 ^{low}	<10%	28%

Supplementary Figure 1. Functional validation of novel mutations. **(A):** RT-PCR of STAT3-target SOCS3 at baseline and in IL-21 activated-EBV cell lines of P3 and P4. Results are shown as relative expression normalized to RPLPO housekeeping gene and represent the mean of 6 independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$ Mann-Whitney test; HC: results pooled from 2 healthy controls (HC1 in green, HC2 in blue), PC: positive control; **(B):** Decreased CTLA-4 mean fluorescence intensity (MFI) on circulating T-cells in P1, P7 and P20 as compared to healthy control (HC) by flow cytometry; **(C):** B-cell immunophenotyping in P8, showing decreased frequency of CD27⁺ and excess of CD21^{low}CD38^{low} B-cells.