Inflammatory Bowel Disease in Patients with Congenital Chloride Diarrhoea

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Inflammatory bowel disease in patients with congenital chloride diarrhoea

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LN: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript.

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Introduction: Congenital chloride diarrhoea (CLD) is a rare autosomal recessive disease caused by mutations in the *solute family carrier 26 member 3* (*SLC26A3*) gene. Patients suffer from life-long watery diarrhea and chloride loss. Inflammatory bowel disease (IBD) has been reported in individual patients with CLD and in *scl26a3*-deficient mice.

Methods: We performed an international multicentre analysis to build a CLD cohort and to identify cases with IBD. We assessed clinical and genetic characteristics of subjects and studied the cumulative incidence of CLD-associated IBD.

Results: In a cohort of 72 patients with CLD caused by 17 different *SLC26A3* mutations, we identified 12 patients (17%) diagnosed with IBD. Nine patients had Crohn's disease, two ulcerative colitis, and one IBD-unclassified (IBD-U). Prevalence of IBD in our cohort of CLD is higher than the highest prevalence of IBD in Europe ($p < 0.0001$). The age of onset was variable (13.5 years, IQR: 8.5 – 23.5 years). Patients with CLD and IBD had lower z-score for height than those without IBD. 4/12 patients had required surgery (ileostomy formation n=2, ileocaecal resection due to ileocaecal valve stenosis n=1, and colectomy due to stage II transverse colon cancer n=1). At last follow-up, 5/12 were on biologics (adalimumab, infliximab, or vedolizumab), 5/12 on immunosuppressant (azathioprine or mercaptopurine), one on 5-ASA and one off-treatment.

Conclusions: A substantial proportion of patients with CLD develop IBD. This suggests potential involvement of SL26A3-mediated anion transport in IBD pathogenesis. Patients with CLD-associated IBD may require surgery for treatment failure or colon cancer.

Key words: Crohn´s disease, ulcerative colitis, SLC26A3, congenital chloride diarrhea, monogenic disease

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Introduction

Congenital Chloride Diarrhoea (CLD; OMIM 214700)[1] was first described in 1945 [2] in a 2-year-old child with congenital watery diarrhoea associated with metabolic alkalosis. This autosomal recessive disorder is caused by mutations in the *solute carrier family 26 member 3* (*SLC26A3*) gene (OMIM: 126650) on chromosome 7q31[3]. Founder mutations from Finland, Poland and Arabic countries have been reported[4,5], along with tens of rare *SLC26A3* mutations, without evidence of genotype-phenotype correlation. The SLC26A3 protein is a major chloride (Cl^-)/ bicarbonate (HCO_3^-) exchanger at the apical brush border of the terminal ileum and colon, the loss of which leads to reduced HCO_3^- secretion and massive chloride loss via the stools[6,7]. As the life-long watery diarrhea starts in utero, CLD pregnancies are characterized by dilated intestinal loops in the fetus, polyhydramnios and preterm birth[8]. Early diagnosis and appropriate salt substitution therapy is associated with a good prognosis, allowing normal growth and development[9]. Intestinal inflammation has been reported in case reports or case series[10–12]. In addition, cellular and animal models support a possible association between *SLC26A3* downregulation and intestinal inflammation, which is probably associated with the impaired NaCl and fluid reabsorption, as well as the absence of a stable intestinal mucus layer[13–15]. Importantly, *SLC26A3* has been identified in a genome-wide association analysis as a potential susceptibility gene for IBD[16].

The aim of this study was to assess the risk of IBD in patients with CLD. We conducted a European survey to identify IBD cases in a large cohort of CLD patients to ascertain clinical and genetic characteristics related to intestinal inflammation.

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Material and methods

Selection of the study cohort

We performed a survey of tertiary European centres of paediatric gastroenterology to collect data from children or adult patients with CLD. Centres from 8 different European countries (Austria, Belgium, Finland, France, Germany, Italy, Poland and the United Kingdom) recorded data. The diagnosis of CLD was based on the clinical characteristics, biochemical markers (faecal chloride concentration) and confirmed by the presence of biallelic *SCL26A3* mutations[4]. Endoscopy is not routinely performed in patients with CLD unless atypical symptoms of CLD appear. Diagnoses of IBD were established according to guidelines for IBD diagnosis[17].

Structured survey

Principal investigators in the participating institutions collected the data and filled in a Case Report Form (CRF) for patients with CLD. We documented CLD characteristic at diagnosis: perinatal history, birth weight, country of living/origin, age and results of diagnostic tests, including genetic analyses. At the most recent follow-up, we collected age, chloride supplementation, height, weight, and body mass index (BMI). In addition, we recorded information on IBD diagnosis (age of onset, follow up period and IBD phenotype, according to the Paris classification[18]), present treatment strategy and surgical history (cause, type, age).

Ethics

Written informed consent was received from all patients participating in IBD cohorts and research projects (such as the Genius cohort of IBD in Paris, the Oxford IBD cohort study, VEO-IBD consortium in Munich, and clinical and genetic studies of CLD in Finland). When

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patients were lost to follow-up, retrospective data were collected from patient charts. All data reported were anonymized, in accordance with protocols approved by the participating institutions.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 21. Categorical variables were compared using Chi Square test and multivariate analysis when appropriate. Correlations were analyzed by Pearson or Spearman test, depending on parametric or non-parametric variables. Proportions are reported with 95% exact binomial confidence intervals (CI). To compare the incidence of IBD in CLD and in the general population, we report risk ratios (RR) as an effect measure, with 95% CI calculated by Fisher's exact test. Age distribution was compared between cohorts with the rank-sum test.

Results

Clinical characteristics of the CLD cohort

Among 76 CLD patients identified, 4/76 were excluded because *SLC26A3* mutation data were not available. A total of 72 patients with full clinical and genotypic characterizations were therefore enrolled (Supplementary table 1). Seventeen different disease-causing mutations in the *SLC26A3* gene were identified. 69/72 patients had homozygous *SLC26A3* variants and three patients were compound heterozygotes (Figure 1). Since the highest proportion (47%) of cases were from Finland (Figure 1), the most frequent mutation was the Finnish founder mutation[6]. The geographical distribution of cases based on their ancestry of origin was 80% European, 16% Middle-East, 3% Asian and 1% African.

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Clinical characteristics of IBD in patients with CLD

In the cohort of 72 patients, 12/72 (17%, 95% CI: 9% - 27%) were diagnosed with IBD. The most frequent presenting symptom that led to diagnosis was bloody diarrhoea in 6/12, with or without iron deficiency anemia in 5/12.

At primary assessment, 11/12 presented with macroscopic and microscopic colonic inflammation at endoscopy; 1/12 had an ileocolic stricture at diagnosis (Figure 2). 8/12 patients were classified as Crohn's disease (CD), 3/12 with ulcerative colitis (UC), and one with IBD-unclassified colitis (IBD-U) according to diagnostic criteria (Table 1). Of note, 1/3 UC patient was reclassified as CD during follow-up.

A comparison between our cohort of 9/12 patients with CD and concomitant CLD and a reference CD population of 900 patients, [19] suggests that isolated colonic involvement in patients with CD and concomitant CLD may be more common (p=0.003).

The diagnosis of IBD was established at a median age of 13.5 years (IQR, 8.5 – 23 years), but a third of patients were diagnosed in the first decade of life, a third in the second decade and the reminder as adults. The age of IBD diagnosis in our small cohort was significantly younger than a reference IBD population[19] (Figure 4). The age-related prevalence of CLD associated IBD is shown in Figure 5.

The 17% prevalence of IBD in our cohort of 72 patients with CLD gives a RR of 17.4 (95%CI: 10.2 – 29.5; $p < 0.0001$) compared to the highest prevalence of IBD reported in Europe[20].

With regard to the impact of both IBD and CLD, having IBD was associated with a lower z-score for height at the most recent follow up visit compared to CLD alone (p=0.03; Table 2).

Perinatal characteristics, such as preterm birth, low birth weight, faecal Cl^- at diagnosis, or amount of Cl^- supplementation (mmol/kg) were not significantly different between patients

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with or without IBD (Table 2). The impact of other therapeutics for CLD, such as butyrate, was not possible because such treatment was not given to of our cohort. There was no significant association between country of origin, ethnicity and type of *SLC26A3* mutation type with IBD development, but numbers are small. Apart from two brothers with concomitant CLD and IBD, no other patients had a family history of IBD.

Treatment of patients with CLD associated-IBD

We recorded the medication of the 12 patients at their most recent follow-up visit (median follow-up 5 yrs, range 2 mo-15 yrs) after IBD diagnosis. The outcomes were as follows. One patient with IBD-U was off treatment after 2.5 years of deep remission (clinical, biological and mucosal healing) on azathioprine. Seven patients were on stable full clinical remission on medical treatment without requiring surgery: one UC patient on 5-ASA, 3 CD patients on azathioprine and 3 (2 CD and 1 UC) on anti-TNFα (1 adalimumab and 2 infliximab). The remnant four CD patients (33%) had undergone surgery (1 total colectomy for stage II colorectal cancer in the transverse colon, 1 de-functioning ileostomy formation, 1 subtotal colectomies with transverse colon stoma and one ileocecal resection for occlusion). The ileocecal resection was performed at the time of IBD diagnosis, while the other three patients underwent surgery more than 10 years after diagnosis (12, 18, and 35 years respectively).

After surgery two had started ustekinumab (anti-TNF failure before surgery) and 2 were off treatment (one azathioprine non-responder in the pre anti-TNFα era and one was infliximab and vedolizumab before surgery).

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Discussion

Our study demonstrates an increased risk of IBD in patients with CLD, suggesting a link between *SLC26A3* downregulation and intestinal inflammation. We observed a 17% prevalence of IBD in our cohort of 72 patients with CLD, which is much higher than the expected population prevalence of IBD in Europe (UC, 505/100,000; CD, 322/100,000 population)[20]. The cumulative incidence of CLD-associated IBD might actually be an underestimate, considering that patients with CLD were younger at last follow up than patients with CLD-IBD. 37% of CLD patients were aged <10 years and 62% were aged <20 years at last follow-up, while the age distribution in our cohort is lower than the distribution in other cohorts with IBD[19]. Growth delay is not only one of the presenting symptoms that should prompt a suspicion of IBD in CLD, but may also persists throughout follow-up.

Our series of patients with CLD is the largest reported, including patients from 8 different countries, caused by 17 different disease-causing *SLC26A3* mutations. Because CLD is an autosomal recessive disease, founder populations such as Finland have a substantial number of heterozygous carriers for *SLC26A3* mutations—the parents of patients with CLD. There is no evidence that heterozygous subjects have any gastrointestinal problems, while heterozygosity for *SLC26A3* variants might be associated with male subfertility[21]. Nevertheless, IBD does not show association with *SLC26A3* variants in genome-wide association analyses among the Finns (data available at http://r4.finngen.fi/). This finding suggests that heterozygous carriers are unlikely to be susceptible to intestinal inflammation, in line with a study on more than 200 IBD risk loci in a large transcriptome data set, which did not find *SLC26A3* as a candidate[22].

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Remarkably, all but one patient in our study had colonic inflammation in association with CLD. In particular, 7 of 8 CD patients in our study had inflammation restricted to the colon (L2). The remaining patient had ileocolic disease (L3), which is the predominant phenotype in paediatric onset CD. None of the 8 patients had upper GI involvement (L4) which occurs in almost 50% of children and adolescents with IBD only[23,24].

Loss of SLC26A3-mediated anion transport in the colon, rather than in the terminal ileum[25–27], might be related to IBD susceptibility in patients with CLD. In line with this, *slc26a3*-deficient mice show an increased risk of mucosal damage and inflammation in the colon[28]. Furthermore, a genome-wide study identified an association between *SLC26A3* and UC[16]. In clinical series, colonic inflammation has been reported in the Finnish cohort of CLD[10], while a study from Saudi Arabia reported no cases of intestinal inflammation[9]. This difference could be due to different age distributions in these CLD series (Finland probably has the oldest survivors[10]), or linked to environmental factors such as diet possibly modulating the gut microbiome[29,30]. Whereas *SLC26A3* deficiency shows a primary association with intestinal inflammation in both humans and mice[31,32], other molecules such as tumor necrosis factor alpha (TNFα) may modulate or downregulate *SCL26A3* expression[33,34].

The importance of ion transport dysregulation in the pathogenesis of IBD is further supported by evidence from animal models[35] and, in humans, by the link between congenital sodium diarrhoea and colonic inflammation[36–38]. This suggests that the pro-inflammatory defect in such patients is epithelial, possibly related to barrier function. Data suggest that changes in the intestinal luminal salt milieu might have a major impact on the intestinal microbiota and IBD susceptibility. Experience in rats and humans[39,40] of diets with

a high salt content, demonstrate alterations in the gut flora with suppression of *Lactobacillus spp*, which can exacerbate colitis. It might be surmised that high faecal chloride in patients with CLD might influence the colonic flora and thereby modulate the risk of colonic inflammation.

In our series, IBD showed a negative effect on the relative height of patients with CLD, no less than it does in other paediatric-onset CD[41].

The severity of CLD-associated IBD is reflected by the need for surgery or biological therapy, although this is not dissimilar to population-based data from Denmark[42] and Finland[43] after more than 5 years from IBD diagnosis.

Limitations linked to the retrospective study design must be acknowledged. An overestimate of the IBD risk cannot be excluded, due to a selection bias from the tertiary care recruitment, but it seems unlikely, since patients with CLD almost invariably require tertiary care.

Our clinical data suggest a link between CLD and IBD, raising the possible role of ion transporters and epithelial dysfunction in the pathogenesis of IBD. Paediatricians and adult gastroenterologists should be alert to a change in the pattern of diarrhea typically associated with CLD, so that appropriate investigation can be performed. Future studies should clarify whether modulating intestinal ion transport[44], microbiota[45,46] or inflammation could be a target to prevent or treat IBD in patients with CLD.

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Conflict of Interest:

No conflict related to this article.

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Table 1: Characteristics of CLD patients with IBD diagnosis

N°	Age CLD diagnosis*	Sex	Genetical mutation	Symptom for IBD suspicion	Age IBD diagnosis°	Grade of colonic inflammation	Phenotype of IBD**	Country of origin/ Ancestry
1	At birth	M	c.1514+1G>T	Abdominal pain, growth delay, spondylarthritis,	12	Moderate	CD (A1bL2B1G1)	France/Africa
2	13	F	917+3_951delGTG	Bloody diarrhea	5	Moderate	UC (E2)	France/Middle East
3	36	M	c.2007+2T>G	Bloody diarrhea, iron deficiency, growth delay	10	Mild	CD (A1bL2B3G1)	Belgium/Caucasian
4	12	M	c.2007+2T>G	Iron deficiency, growth delay	13	Moderate	CD (A1bL2B1G1)	Belgium/Caucasian
5	33	M	c.559G>T	Persistent iron deficiency anemia	8	Moderate	CD (A1bL2B1G0)	UK/Middle East
6	12	F	c.2024_2026dup	Bloody diarrhea	1	Mild	IBD-U (E3)	UK/Caucasian
7	166	F	C.272 _971 del	Bloody diarrhea	14	Mild	UC (E3)	Austria/Caucasian
8	At birth	F	c.949_951delGTG	Bloody diarrhea	34	Moderate	CD (A2L2B1)	Finland/Caucasian
9	At birth	F	c.949_951delGTG	Intestinal occlusion	25	No inflammation but stenosis	CD (A2L3B2)	Finland/Caucasian
10	At birth	F	c.949_951delGTG	Fever, erythema nodosum	30	Moderate	CD (A2L2B3p)	Finland/Caucasian
11	At birth	F	c.949_951delGTG	Bloody diarrhea, anemia Elevation of ESR, episodic fever,	17	Unknown	First diagnosed UC (E3) then CD at age 25 (A2L2B3p)	Finland/Caucasian
12	At birth	F	c.949_951delGTG	Anemia	17	Moderate	CD (A2L3B1)	Finland/Caucasian

*expressed in months
°expressed in years
** expressed in: Crohn Disease CD, Ulcerative Colitis UC Inflammatory Bowel Disease Unclassified IBD-U (Paris Classification)[18]
IBD: Inflammatory Bowel Disease
CLD: Congenital Chloride Diarrhea

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Table 2: Comparison between patients with CLD alone and IBD and CLD

	CLD no-IBD	CLD-IBD	P
Sex (female)	25/54 (46%)	6/11 (54%)	0.61
Preterm birth	39/53 (74%)	8/10 (80%)	0.66
Intrauterine growth retardation	8/49 (16%)	2/10 (20%)	0.77
Mutation type:			
Deletion	38/60 (63%)	7/12 (59%)	
Nonsense	10/60 (17%)	1/12 (8%)	
Splice-site change	4/60 (7%)	3/12 (25%)	
Insertion	6/60 (10%)	1/12 (8%)	
Missense	2/60 (3%)	0/12	0.35
Age at last follow-up	13 (6.5-30.5)	23 (9.5- 37)	0.16
Cl supplementation at last follow-up (mEq/kg)	2.3 (1-3)	4.8 (1.1-5.4)	0.16
Weight at last follow-up (z-score)	0.71 (-0.4-1.3)	-0.13 (-1.8-0.4)	0.07
Height at last follow-up (z-score)	0.3 (-0.4-1.1)	-0.46 (-1.8-0.05)	0.03*
BMI at last follow-up (z-score)	0.79 (-0.4-1.4)	0.15 (-1-0.7)	0.17

* Significant for P < 0.05

CLD: Congenital Chloride Diarrhea, IBD: Inflammatory Bowel Disease, BMI: Body Mass Index

Values are expressed as proportion (percentage) or median (interquartile range)

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Figure 1: Mutation on the SCL26A3 gene

Representation of different pathogenic germline SCL26A3 mutations in 69 patients with CLD investigated. Symbols represent the mutation site of individual patients. Coloured symbols represent patients who present with IBD phenotype.
* Nonsense mutations account for Saudi Arabia founder mutations
** Deletion mutations account for Finnish founder mutations
*** Insertion mutation account for Polish founder mutations

Figure 2: Colonic and ileocolic inflammation in patient's whit concomitant CD and CLD

Figure 3: Age distribution of IBD in patients with Congenital Chloride Diarrhoea compared to distribution in general population

Figure 4: Curve of penetrance of IBD phenotype in CLD patients

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Figure 1

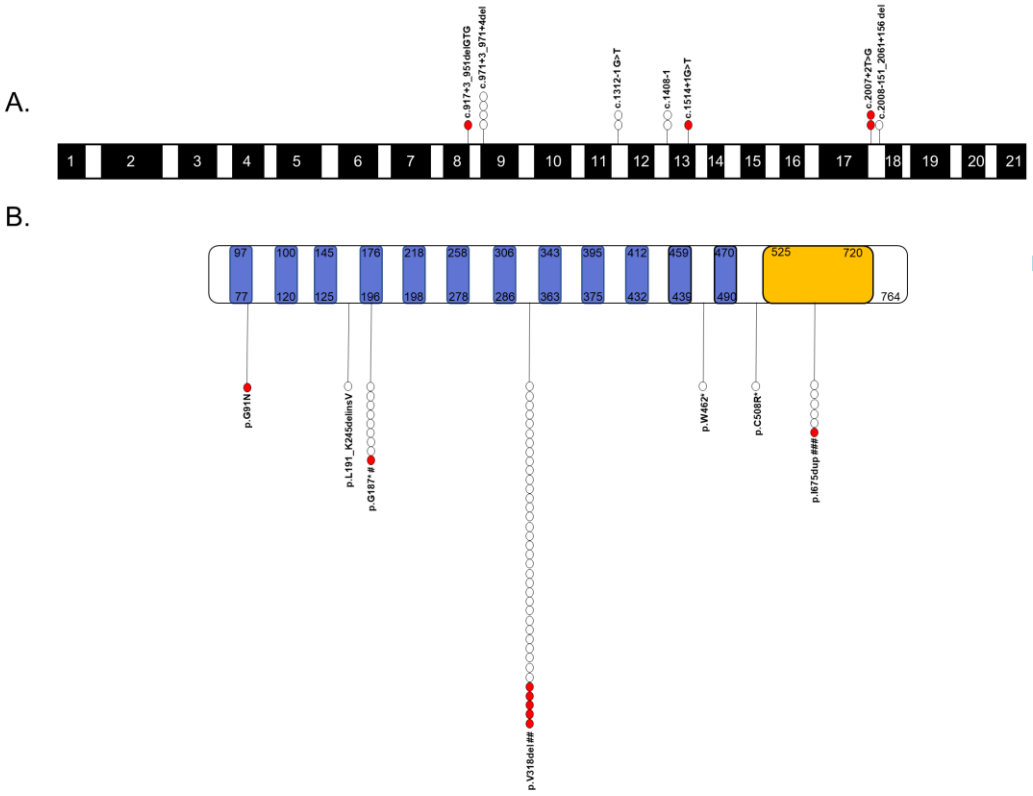

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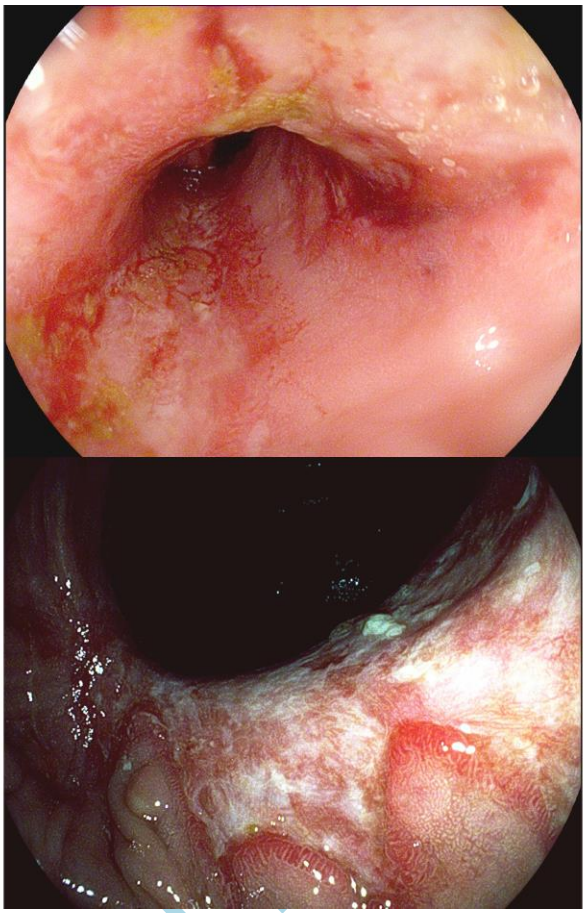
Figure 2



Manuscript Doi: 10.1093/ecco-jcc/jjab056
Figure 3

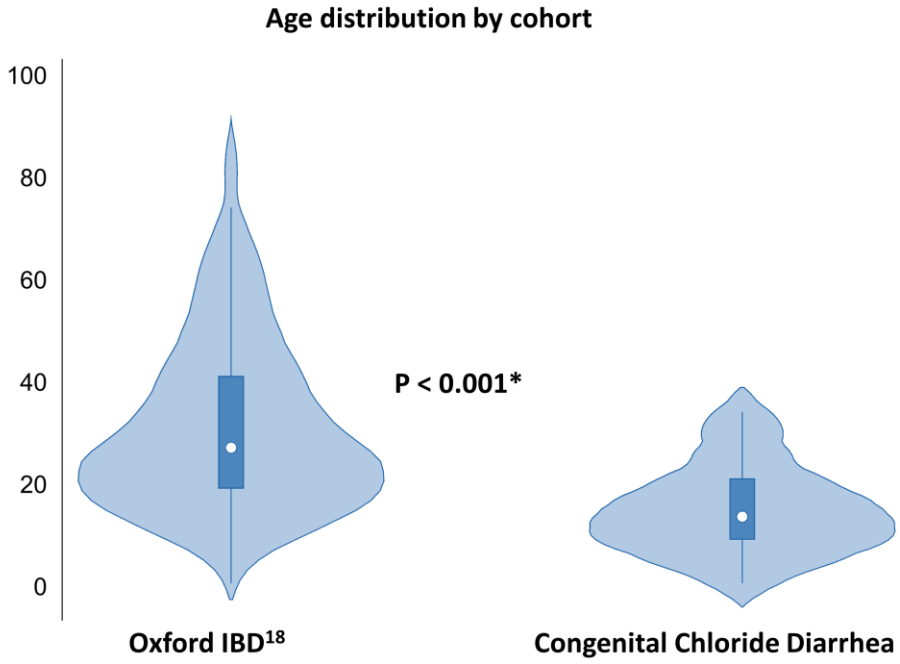


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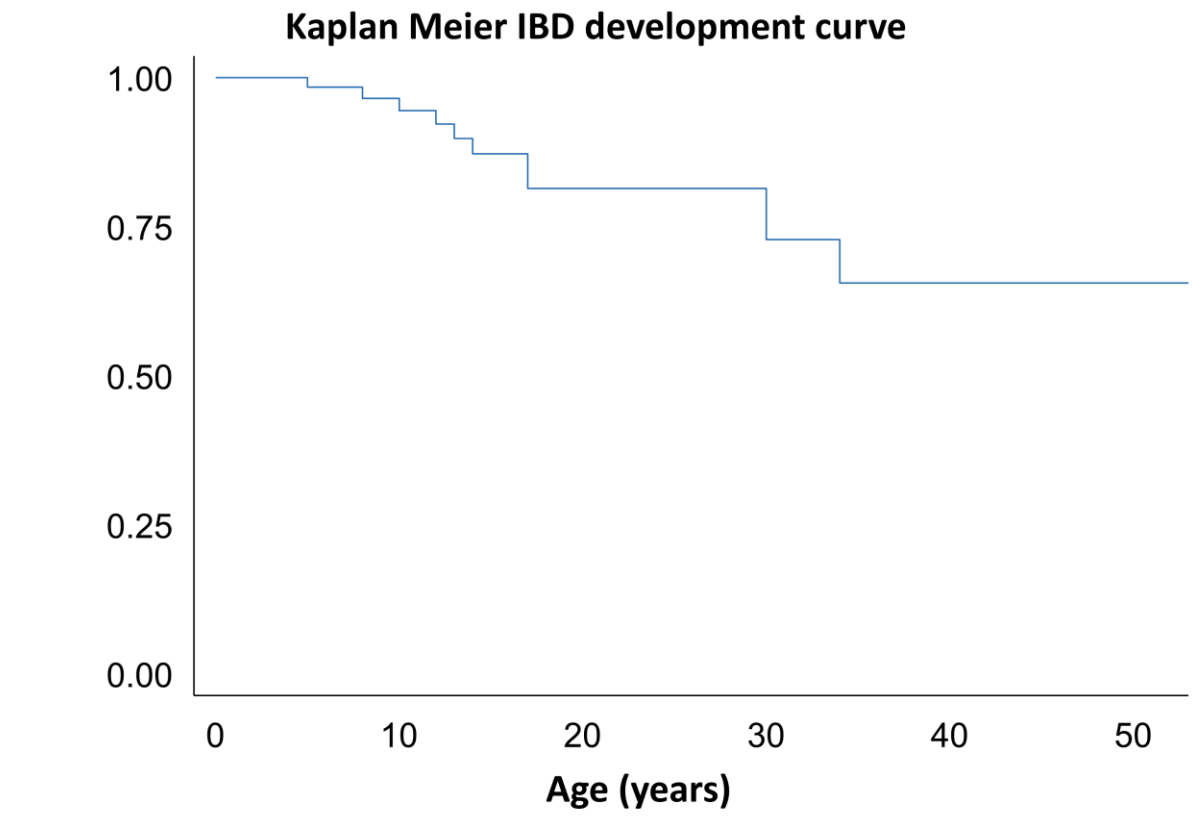
Figure 4



Number at risk:	72	44	26	19	4	1
New IBD:	2	6	0	4	0	0