Ileal Involvement Is Age Dependent in Pediatric Crohn's Disease

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Background: Lymphoid follicles (LFs) have been suggested to play a role at the early stage of Crohn's disease (CD) lesions. In the small bowel, LFs are grouped, forming Peyer's patches, which develop early in fetal life, grow in size and number until puberty, and undergo involution. In contrast, colonic LFs are isolated and undergo little change during life. As a result, if LFs play a role in the occurrence of CD lesions, the distribution of ileal and colonic lesions is expected to be altered in small children.

Methods: Medical records of 2 independent French (n = 136) and Swedish (n = 55) cohorts of consecutive pediatric CD were reviewed. Disease sites and age of onset were recorded, and the age-dependent probability to develop ileal lesions was computed. The CARD15/NOD2 genotype was also analyzed when available (n = 99).

Results: The curves of disease occurrence were significantly different in case of CD with or without ileal lesions ($P < 0.0001$). At the age of 8 years, the probability (95% confidence interval) of small bowel involvement was 0.19 (0.07–0.39). It increased until 16 years of age to 0.61 (0.54–0.68). It was slightly higher in patients carrying 1 or more *CARD15/NOD2* mutations [0.75 (0.55–0.89)] than in wild-type patients [0.46 (0.34–0.58)]. *CARD15* mutations also influenced the age of onset of ileal disease ($P < 0.02$).

Conclusions: In children, ileal CD lesions are delayed compared with colonic lesions. This observation is in agreement with the previously proposed hypothesis of a pathophysiological role of Peyer's patches in ileal CD. The rarity of small bowel lesions should be a warning to be cautious when classifying chronic colitis in small children.

Key Words: CARD15, children, Crohn's disease, lymphoid follicle, NOD2, Peyer's patches

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Crohn's disease (CD) is an immune-mediated chronic inflammatory disorder of the digestive tract. Its pathogenesis and etiology are largely unknown, but the interaction between gut-associated immune cells and intestinal bacteria seems to play an important role for initiation and perpetuation of the chronic inflammation.[1–4] For example, in animal models of inflammatory bowel disease (IBD), the inflammation often occurs only in presence of microorganisms.[2,3] Another strong argument supporting this point of view has been provided by the discovery of an association between *CARD15/NOD2* mutations and CD.[5–7] CARD15/NOD2 encodes for an intracellular pattern recognition receptor of the innate immune system that recognizes a peptidoglycan component—the muramyl di-peptide—present in both gram-negative and gram-positive bacteria. However, the biologic effects of the mutations associated to CD are still subject to debate. While a defective nuclear factor-κB–mediated production of proinflammatory cytokines is usually suggested to have a key role,[8] recent publications alternatively suggest that CD mutations are characterized by an excess of T-helper 1 (T_H1) cytokine production[9] or an excess of intereukin-1β production.[10] Despite these differences, the final consequence of the biologic alterations consists in recruitment of cells involved in adaptive immunity and chronic inflammation of the digestive tract.

In the gut, specific lymphoid formations, known as lymphoid follicles (LFs), play a key role in the host–bacteria interaction. LFs are mainly encountered in the colon, where they are isolated, and in small bowel, where they are grouped,

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forming Peyer's patches (PPs).[11–15] Several arguments suggest that LFs are important in CD lesion formation: (1) CD lesions are usually localized in colon and distal ileum where the LFs are the most abundant[16–18]; (2) they have a discontinuous distribution as well as PPs and LFs; and (3) they seem to be centered by LFs using electron microscopy analyses.[15]

In addition to this spacial relationship between CD lesions and LFs, a temporal link between CD and PP development has also been suggested.[19] In the small bowel, PPs develop early in fetal life, grow in size and number until puberty, and then undergo involution.[16] After a peak occurring between 15 and 20 years of age, with an average number of 240, only 100 PPs remain after the age of 70 years.[16] Van Kruiningen et al[19] compared the curve of PP abundance and the curve of CD incidence. CD increases in frequency in teenagers, with a peak in the third decade, followed by a decrease thereafter. As a result, the 2 curves seem parallel, with a delay of 5 to 10 years between the peaks of CD incidence and PP number. Such a delay could be explained by the necessity of a chronic stimulation by microorganisms before the formation of the CD lesions and/or by a delay between the development of CD lesions and the occurrence of clinical symptoms.

In the colon, the concentration of LFs is less subject to temporal variations than in the ileum.[20] Following the hypothesis of an inaugural role of LFs in CD lesions, we can thus postulate that colonic lesions are less subject to lifelong temporal changes. As a result, the ratio ileitis/colitis must be lower in young children and seniors. Ileitis has been reported to be less frequent in seniors than in young adults, confirming this idea.[21] In contrast, the question has not been fully documented in children. For this purpose, we have analyzed 2 independent cohorts of French and Swedish pediatric CD cases.

MATERIALS AND METHODS

Patients

We retrospectively studied medical records of all consecutive pediatric CD cases followed at Robert Debré Hospital in Paris, France, between January 1999 and December 2003 and at the Astrid Lindgren's Children Hospital in Stockholm, Sweden, between October 1992 and August 2002. In total, we identified 198 incident patients younger than 16 at the onset of the disease. Medical records were reviewed regarding age at onset of symptoms, age at diagnosis, location of lesions at diagnosis, and diagnostic criteria. Seven patients were excluded because medical records were incomplete concerning initial disease location. The remaining 191 patients (136 in Paris and 55 in Stockholm) were included in this study. For comparison between the 2 cohorts of patients see Table 1.

TABLE 1. Comparison Between the Paris and Stockholm Cohorts of Patients with CD

	Paris	Stockholm
No. of patients	136	55
Sex ratio (M/F)	1.0	1.5
Time interval of data recruitment	1999–2003	1992–2002
Median age at diagnosis (months [25%–75% CI])	12.0 [10.3–13.6]	11.8 [9.9–13.4]
Median diagnostic delay (months [25%–75% CI])	6.0 [2.5–12.0]	3.0 [2.0–7.0]
Initial localization		
Isolated ileal [n (%)]	8 (6%)	4 (7%)
Isolated colonic [n (%)]	42 (31%)	31 (56%)
Colonic and ileal [n (%)]	87 (63%)	20 (36%)
CARD15 mutated/genotyped patients [n (%)]	24/44 (55%)	4/55 (7%)

Data Recording

CD diagnoses were established according to international criteria based on clinical, histologic, endoscopic, and radiologic data.[22] Ulcerative colitis (UC) and indeterminate colitis were not taken into account in the analyses.

CD ileitis was retained in case of involvement of the ileum detected by small bowel follow-through, tomodensitometry imagery, endoscopy, and/or histology. In fact, ileoscopy was available in most of patients, and the diagnosis of ileitis was rarely (n = 3) based on radiologic findings only.

In case of colonic lesions, CD was retained if 1 or more of the following criteria were present at onset or during follow-up: (1) presence of granuloma formations and/or giant cells at histology; (2) association with endoscopic and/or histologic lesions compatible with CD in the upper digestive tract; (3) association with perianal lesions including fistulas, abscesses, and deep fissures; (4) discontinuous colonic lesions and/or no rectal involvement at endoscopy and histology; and (5) presence of intestinal strictures, abscesses, or fistulas. The diagnosis of CD ileocolitis was retained in case of ileal CD associated with colonic lesions.

Because date of onset and date of diagnosis may differ, we recorded both. Date of onset was defined by the first time when the patient experienced symptoms attributable to CD. Date of diagnosis was defined by the date when a doctor first mentioned the diagnosis of CD. This date was usually the date of the first colonoscopy. Patients with a final diagnosis of CD but an inaugural classification as indeterminate colitis were included. In these cases, the date of the initial diagnosis was retained as the date of diagnosis.

Genotyping Data

CARD15/NOD2 genotypes were available in a subgroup of 99 patients (44 in Paris and 55 in Stockholm) who partially contributed to a previously published study.[23] Genotyped patients were classified as mutation carriers or noncarriers

according to the presence or absence of the 3 main CD-associated mutations (*R702W*, *G908R,* and *1007fs*) in their genotype.

Statistical Analyses

Quantitative variables were expressed as medians (first to third quartiles) and qualitative variables as frequencies (95% confidence interval). Time of CD occurrence was displayed by the mean of the 1 minus Kaplan-Meier curve. Because no observations were censored, median times of occurrence of CD depending on the initial localization and the carriage of 1 or more *CARD15/NOD2* mutations were compared by the Wilcoxon-rank sum test. All statistical tests were 2-tailed. Statistical analysis was performed using SAS 8.02 (SAS, Cary, N.C.) and Splus 6.2 (MathSoft, Seattle, Wash.) software packages for PC.

RESULTS

In our study group, the male/female sex ratio was equal to 1.0 in Paris, 1.5 in Stockholm, and 1.1 for pooled cohorts. The median (25%–75% confidence interval) age at diagnosis was not significantly different in Paris (12.0 [10.3–13.6] yrs) and Stockholm (11.8 [9.9–13.4] yrs). Median diagnostic delay was also comparable between the 2 cohorts: 6.0 (2.5–12.0) months in Paris and 3.0 (2.0–7.0) months in Stockholm (Table 1).

Patients were divided into 3 groups according to the initial location of CD lesions: ileal, ileocolonic, and colonic diseases. Ileal disease was found in 12 patients, ileocolonic disease in 106 patients, and isolated colonic disease in 73 patients (Table 1). Because of the small number of patients with isolated ileal disease, patients with ileal and ileocolonic disease were pooled. The diagnostic delay did not differ between patients with ileal or ileocolonic disease (7.0 [3.0–16.0] mo) and patients with colonic disease (5.0 [2.0–8.0] months).

Age of diagnosis according to initial localization was computed for each geographic group (Fig. 1, A and B) and for the pooled cohort (Fig. 1C). In these cohorts, colonic location occurred as early as the age of 1 year, whereas the youngest child with an ileal location was 5 years old. In both countries, most of the youngest patients show an involvement of the large bowel only. In fact, small bowel involvement occurred mainly in children older than 8 or 10 years (Fig. 1C).

For the pooled cohort, the median age of diagnostic was 11 [8–13] years in the group without ileal involvement and 12 [10–14] years in the group with ileal disease. The Wilcoxon rank sum test comparing the 2 Kaplan-Meier curves was highly significant ($P < 0.0001$).

Finally, we computed the probability (95% confidence interval) of inaugural ileal lesions conditionally to the age at diagnosis (Table 2). This probability increases from 0.0 (0.0–0.48) at the age of 5 years to 0.61 (0.54–0.68) at the age of 16 years, with a dramatic change between the age of 8 (0.19 [0.07–0.39]) and 12 years (0.57 [0.48–0.66]).

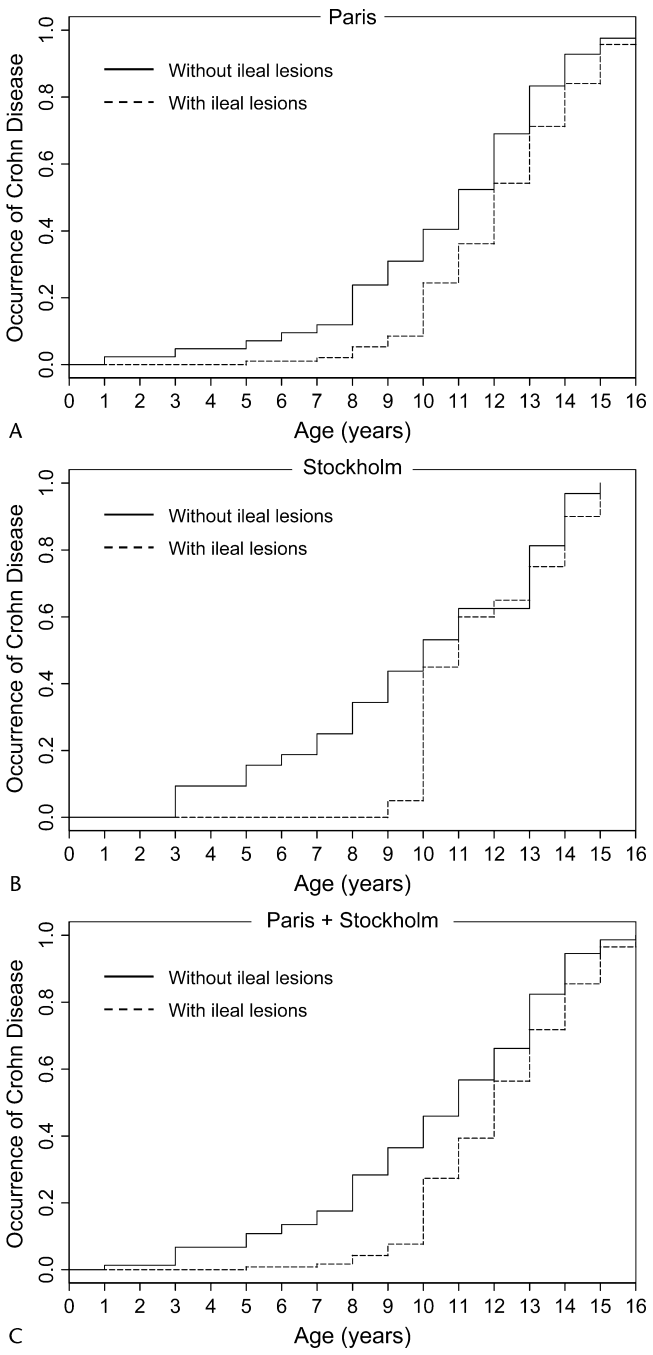

FIGURE 1. Kaplan-Meier curve showing the occurrence of colon only (solid line) and ileal (dashed line) CD in pediatric patients. The results from the French (A), Swedish (B), and pooled (C) cohorts are shown.

Genotypes were available in 45 patients with colonic involvement and 54 patients with ileal or ileocolonic involvement. The conditional probability for developing a small bowel disease at 16 years was slightly higher in patients carrying 1 or more *CARD15/NOD2* mutations (0.75 [0.55–0.89]) than in wild-type patients (0.46 [0.34–0.58]). In the group of

TABLE 2. Cumulative Probability of Ileal Lesions in Pediatric CD

Age	No. of Patients	Patients with Ileal Lesions	Probability (95% CI) of Ileal Lesions
≤1 yr	1	0	0.00
≤2 yrs	1	0	0.00
≤3 yrs	5	0	0.00 (0.00–0.52)
≤4 yrs	5	0	0.00 (0.00–0.52)
≤5 yrs	9	1	0.00 (0.00–0.48)
≤6 yrs	11	1	0.00 (0.00–0.41)
≤7 yrs	15	2	0.13 (0.02–0.41)
≤8 yrs	26	5	0.19 (0.07–0.39)
≤9 yrs	36	9	0.25 (0.12–0.42)
≤10 yrs	66	32	0.48 (0.36–0.60)
≤11 yrs	88	46	0.52 (0.42–0.62)
≤12 yrs	115	66	0.57 (0.48–0.66)
≤13 yrs	145	84	0.58 (0.50–0.66)
≤14 yrs	170	100	0.59 (0.51–0.66)
≤15 yrs	186	113	0.61 (0.54–0.68)
≤16 yrs	191	117	0.61 (0.54–0.68)

patients with ileal involvement, the Kaplan-Meier curves were significantly different between patients with or without mutations (Fig. 2; $P < 0.02$).

DISCUSSION

The annual incidence of pediatric CD has recently been estimated at $2.3/10^5$ children in France[24] and $4.9/10^5$ children in northern Stockholm.[25] The disease may affect all parts of the digestive tract from mouth to anus, including the small bowel and colon. In this work, we analyzed the relative frequency of ileal and colonic involvements in 2 independent cohorts of children followed in specialized centers of pediatric gastroenterology in Sweden and France. Our data support the conclusion that ileal CD occurs rarely before the age of 8 years and increases thereafter until adulthood.

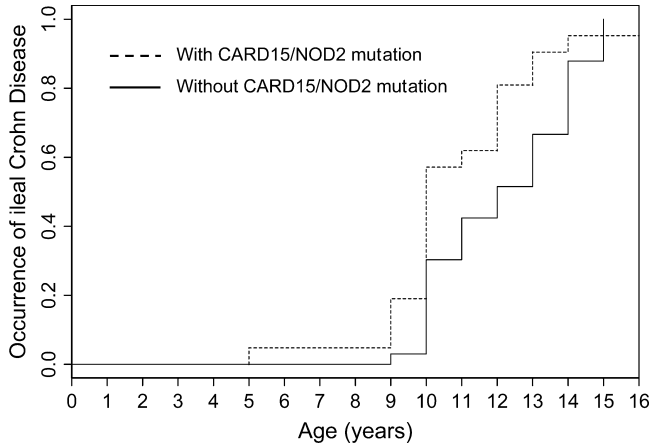

FIGURE 2. Kaplan-Meier curve showing the occurrence of ileal lesions in patients with CD with (dashed line) or without (solid line) a *CARD15/NOD2* mutation.

This study is a retrospective analysis based on public hospital cohorts. Both French and Swedish health care systems are characterized by easy access to good-quality hospitals. It is thus unexpected that a significant proportion of children with CD remain undiagnosed for a long period. The limited delays (3 and 6 mo, respectively) between the first symptoms and the diagnosis support this opinion.

It can also be argued that the studied populations reflect a particular subgroup of patients with CD. The most severe diseases are usually referred to public hospitals, but recruitment biases may be more difficult to discard for less severe diseases. However, several lines of evidence suggest that large biases are unexpected. In big cities such as Paris and Stockholm, gastroenterologists do not perform routine colonoscopy in children, and they do not usually follow up with the youngest patients. As a result, we can postulate that most pediatric cases of CD are seen by pediatric gastroenterologists. However, to limit putative recruitment biases in older children, we chose to limit the study to children younger than 16 years. Finally, the same conclusions could be drawn when lowering the age at onset for the definition of pediatric cases (see Table 2). In conclusion, we do not think that recruitment biases significantly altered the main conclusion of the study.

CD is rare in very young patients: only 26 patients were diagnosed before their 9th birthday, whereas 165 children were diagnosed between their 9th and 16th birthdays. This rarity of CD in very young people suggests that CD lesions and symptoms appear progressively with time, that patients are exposed to environmental factors only after the first years of life, and/or that there is a critical age for disease emergence. The above described LF hypothesis can be placed in the third (non exclusive) category.

Pediatric CD is characterized by a large proportion of colonic involvement. In fact, ileitis is only rarely found in pediatric cases: only 12 of 191 cases were identified in our 2 cohorts. In contrast, CD ileitis is usually associated with colitis. This observation suggests that ileitis does not occur independent of colitis, arguing for a common disease mechanism for the 2 locations of the disease in children.

Our main observation was that CD in young children rarely affects the ileum, whereas the colon may be affected in all age groups. In fact, small bowel involvement usually occurs only after the age of 10 years. This observation was made on 2 independent cohorts of patients. In this work we have only included patients with CD using stringent criteria to avoid a "contamination" by indeterminate or UC. It is therefore unlikely that these results could be explained by a misclassification of the disease. In addition, even if present, such a misclassification is not expected to affect the comparison

between age groups. In fact, analyses performed using the whole IBD phenotype showed an even more noticeable difference between ileal and colonic diseases (data not shown).

Case reports of infants with CD do not usually find ileal involvement.[26–29] A more comprehensive study describing IBD in children 5 years of age and younger found ileal disease in 14/35 (40%) patients with a final diagnosis of CD.[30] This value is within the confidence interval of our risk estimate for this age group (0.01–0.48). It is lower than the value observed in older children,[31] adolescents,[32] and young adults,[21] confirming our observation. Another finding in accordance with our data came during the preparation of this manuscript from an analysis of a pediatric IBD consortium registry reporting that isolated colonic CD disease tends to be more frequent in children younger than 6 years than in children and adolescents.[33]

Values of ileal involvement frequencies higher than the present ones have been published in children. Grybowski[31] studied CD in children 10 years old or younger. The authors found isolated ileal or combined ileal and colonic localization in 60% of these patients. This value is at the upper limit of our confidence interval for this age group (0.36–0.61). A meta-analysis of 1153 patients from 12 pediatric centers (all ages confounded) showed small bowel involvement with or without colonic involvement in 76% of the patients.[32] This value is higher than our own data (0.61 [0.54–0.68]). However, despite these observed discrepancies with our data, the same conclusion of a defect of ileal involvement in the youngest children can be drawn when comparing these 2 reports.

In this study, we looked only at the inaugural CD lesions, but it is also interesting to look at the secondary occurrence of ileal disease in patients with an inaugural colitis. Gryboski[31] working with children with CD under the age of 10, found that 5 of 12 patients (41%) presenting initially with an isolated colonic disease developed ileal lesions 6 months to 5 years later. In Paris, 8 of the 74 patients (11%) presenting initially with isolated large bowel disease developed small bowel lesions during the course of disease. Delayed small bowel involvement in children is an additional argument suggesting that ileal lesions are age-dependent.

Small bowel involvement is an important feature distinguishing UC from CD. We showed that the cumulative probability for finding small bowel involvement in the youngest patients with CD is low (e.g., the probability is 0.19 [0.07–0.39] at the age of 8 years). As a result, in most of the youngest patients with CD, ileal involvement is lacking, and CD diagnosis is only based on colonic lesions. The diagnosis of CD before the age of 10 years may thus be more difficult than in older patients, justifying precautions when classifying UC as chronic colitis in this age group. Consistent with this position is the observation reported by Mamula et al[30] that the diagnosis of CD in patients younger than 5 years was made after several years of evolution in nearly 20% of cases.

In this study, the proportion of patients with CD with mutations was 24 of 44 French patients and 4 of 55 Swedish patients. This geographic difference can be explained by a heterogeneous distribution of *CARD15/NOD2* mutations in Europe with a south-north gradient in Europe.[34] *CARD15* mutations are known to influence disease location, and a UC-like phenotype is very rare in people carrying *CARD15/NOD2* mutations on their 2 chromosomes.[23] As a result, ileal involvement (associated or not with colonic involvement) is the rule in CD patients with mutations. In our study, CARD15/NOD2 status influenced disease location. In addition, we confirmed previous reports that it also influences the age of onset of ileal disease.[23]

In conclusion, our study showed that the cumulative probability of ileal CD increases with age until puberty. This observation supports the hypothesis of a relationship between LFs and CD lesions. From a clinical point of view, the rarity of small bowel involvement in the youngest patients warns clinicians to be cautious when classifying chronic colitis in young children.

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