

The significance of ileo-colonic lymphoid nodular hyperplasia in children with autistic spectrum disorder

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Background Intestinal mucosal pathology, characterized by ileo-colonic lymphoid nodular hyperplasia (LNH) and mild acute and chronic inflammation of the colorectum, small bowel and stomach, has been reported in children with autistic spectrum disorder (ASD).

Aim To assess ileo-colonic LNH in ASD and control children and to test the hypothesis that there is an association between ileo-colonic LNH and ASD in children.

Patients and methods One hundred and forty-eight consecutive children with ASD (median age 6 years; range 2–16; 127 male) with gastrointestinal symptoms were investigated by ileo-colonoscopy. Macroscopic and histological features were scored and compared with 30 developmentally normal (non-inflammatory bowel disease, non-coeliac disease) controls (median age 7 years; range 1–11; 25 male) showing mild non-specific colitis in 16 cases (13 male) and normal colonic histology in 14 cases (12 male). Seventy-four ASD children and 23 controls also underwent upper gastrointestinal endoscopy. The influence on ileal LNH of dietary restriction, age at colonoscopy, and co-existent LNH elsewhere in the intestine, was examined.

Results The prevalence of LNH was significantly greater in ASD children compared with controls in the ileum (129/144 (90%) vs. 8/27 (30%), $P < 0.0001$) and colon (88/148 (59%) vs. 7/30 (23%), $P = 0.0003$), whether or not controls had co-existent colonic inflammation. The severity of ileal LNH was significantly greater in ASD children compared with controls, with moderate to severe ileal LNH present in

98 of 144 (68%) ASD children versus 4 of 27 (15%) controls ($P < 0.0001$). Severe ileal LNH was associated with co-existent colonic LNH in ASD children ($P = 0.01$). The presence and severity of ileal LNH was not influenced by either diet or age at colonoscopy ($P = 0.2$). Isolated ileal LNH without evidence of pathology elsewhere in the intestine was a rare event, occurring in less than 3% of children overall. On histopathological examination, hyperplastic lymphoid follicles are significantly more prevalent in the ileum of ASD children (84/138; 61%) compared with controls (2/23; 9%, $P = 0.0001$).

Conclusion Ileo-colonic LNH is a characteristic pathological finding in children with ASD and gastrointestinal symptoms, and is associated with mucosal inflammation. Differences in age at colonoscopy and diet do not account for these changes. The data support the hypothesis that LNH is a significant pathological finding in ASD children. *Eur J Gastroenterol Hepatol* 17:827–836
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Introduction

We have presented evidence for the existence of a complex disorder comprising ileo-colonic lymphoid nodular hyperplasia (LNH) and mild acute and chronic inflammatory mucosal pathology in a subset of children with developmental disorders [1,2]. The majority of children were referred with a diagnosis of autistic spectrum disorder (ASD), often described as atypical or regressive autism [1,2]. The intestinal lesion, which has now been characterized in previous work [3,4] against appropriate controls, using a combination of semi-quantitative histochemistry, quantitative immunohistochemistry, and flow cytometric analysis of the mucosal lymphocyte infiltrate by cell phenotype and intracellular

cytokine profile, appears to represent a novel enterocolitis (autistic enterocolitis). Briefly, the lesion is associated with the endoscopic features of often florid LNH with mucosal erythema, loss of vascular pattern, and occasional aphthoid ulceration. Histologically, the lesion is patchy, and appears to be confined to the mucosa where it is associated with specific immunopathology [3,4]. In the colonic mucosa, $\gamma\delta$ T-cell and CD8⁺ densities, and basement membrane thickness were significantly increased in ASD children compared with histologically normal and inflammatory bowel disease (IBD) controls [3]. In the upper gastrointestinal tract, duodenal intra-epithelial and lamina propria CD8⁺ lymphocyte densities, and crypt cell proliferation were increased in ASD

children compared with controls [3]. The pattern of gastrointestinal pathology, including ileo-colonic LNH and deposition of immune complex in the duodenal epithelial basolateral membrane, has been considered to have features of an auto-immune disorder [4].

There is an anecdotal impression that ileo-colonic LNH is a normal finding in children undergoing radiographic and endoscopic investigation for gastrointestinal symptoms [5], with colonic LNH being more frequent in younger children [6–10]. However, normal children, as opposed to children with symptoms indicative of underlying pathology, are not subjected to these procedures. If LNH is the only finding at such an examination it may be the source of such symptoms. On the other hand, systematic endoscopic, histopathological, and immunological studies indicate that ileo-colonic LNH is a significant feature in children with lower gastrointestinal bleeding [11] and food allergy [12,13] and, in a broader age range, patients with immune deficiencies and persistent microbial infections [14–18]. The relevance of these factors to the pathogenesis, and the influence of age upon the prevalence and degree of the chronic LNH in ASD children need to be considered.

Many ASD children are on gluten and casein exclusion diets and behavioural improvements have been reported as a consequence [19]. The rationale for diet includes the removal of precursors for exorphins with their potential for neurotoxicity [20]. In addition, the potential for an effect of these diets on the associated intestinal lesion also merits consideration, given the immunogenic potential for gluten and casein in the gastrointestinal mucosa.

Previously, we have reported a significantly increased prevalence of ileo-colonic LNH in ASD children compared with children with a typical IBD (ulcerative colitis) and children with histologically normal colonic mucosa [2]. In the present study we have examined prospectively a substantially larger group of ASD children comparing these findings with those in children with non-specific colonic inflammation. We have also examined the effect of diet and age upon the prevalence of ileo-colonic LNH. We test the hypothesis that ileo-colonic LNH is a significant pathological finding in ASD children.

Table 1 Characteristics of groups of autistic spectrum disorder (ASD) children and developmentally normal controls undergoing ileo-colonoscopy

Group	Age (years)	Number (male gender)	Children undergoing upper GI endoscopy
ASD children	6 (2–16)	148 (127)	74 (50%)
All developmentally normal controls	7 (1–11)	30 (25)	23 (77%)
Developmentally normal controls (with colonic pathology)	7.5 (1–11)	16 (13)	15 (94%)
Developmentally normal controls (no colonic pathology)	6.3 (1–11)	14 (12)	8 (57%)

n = number of cases. Ages = median (range).

Patients and methods

The study is based upon data collected prospectively from a cohort of 148 consecutively investigated children with developmental disorder on the autistic spectrum (from here on termed 'ASD children') (Table 1) and a control cohort comprised of 30 developmentally normal children (Table 1). There were no statistically significant differences in age and gender between the two groups. The group of developmentally normal children included those in whom the final diagnosis was of mild non-specific inflammatory changes (Table 1) or normal colonic histology (Table 1). All ASD and control children were selected from the same source population, that is, children referred to the Centre for Paediatric Gastroenterology, Royal Free Hampstead NHS Trust, UK, for investigation of gastrointestinal symptoms according to clinical need. All children were referred for investigation by either their general practitioner or paediatrician, and were investigated as inpatients between 1996 and 2001. Children were investigated consecutively in order to avoid selection bias. As part of our continuing studies of gastrointestinal problems in ASD children, both children investigated and reported previously [2] and later consecutive cases were analysed in view of the novel hypotheses under investigation. The rate of false negative pathology in this unit is low. Accordingly, only a limited number of endoscopies with normal findings were undertaken during this period, and consequently the number of control endoscopies is, by default, relatively small. Investigation of children as part of these studies was approved by the Ethical Practices Committee of the Royal Free Hampstead NHS Trust.

Inclusion criteria for affected children included: gastrointestinal symptoms (summarized in Table 2) sufficient

Table 2 Symptoms in the ASD and control groups

Symptoms	ASD (<i>n</i> = 148)	Developmentally normal controls (<i>n</i> = 30)
Constipation	75 (51)	4 (13)
Diarrhoea/loose stool	45 (30)	8 (27)
Abdominal pain/diarrhoea/constipation	19 (13)	0 (0)
Abdominal pain	6 (4)	14 (47)
Rectal bleeding	0 (0)	4 (13)
Other	3 (2)	0 (0)

n = number of cases in symptom group (%).

Table 3 Details of dietary restrictions

Diet	ASD children (n=148)	All controls (n=30)	Developmentally normal controls with colonic pathology (n=16)	Developmentally normal with no colonic pathology (n=14)
Normal diet	68 (50)*	28 (94)	15 (94)	13 (93)
Gluten free diet	17 (12)	1 (3)	1 (6)	0 (0)
Dairy/casein free diet	24 (18)	1 (3)	0 (0)	1 (7)
Dairy/casein + gluten free diet	28 (20)	0 (0)	0 (0)	0 (0)
No dietary history available	11	0 (0)	0 (0)	0 (0)

Number on diet (n) with (% on the diet out of the number of the 137 ASD cases with a known dietary history).

*Pica was seen in one case but the patient was on an otherwise normal diet.

to warrant invasive investigation; a developmental disorder on the autistic spectrum diagnosed according to DSM-III-R/ICD-10 criteria, where no other exclusive cause for their developmental disorder had been identified e.g. fragile X; there was no contraindication to anaesthetic for endoscopy, and written, fully informed parental consent was obtained for the procedure. In ASD children, consent to the procedure was not declined in any case. All developmental diagnoses in ASD children were made prior to referral for a gastroenterological opinion, by a suitably qualified psychiatrist, psychologist or developmental paediatrician. Inclusion criteria for controls included gastrointestinal symptoms sufficient to merit ileo-colonoscopy; no history of a developmental disorder; there was no contraindication to anaesthetic for endoscopy, and written parental consent was obtained for the endoscopic procedure.

In 137 (93%) of the ASD children and all controls, a dietary history (including dietary restrictions at the time of endoscopy) was obtained from the clinical records and cross-checked with the inpatient nursing records (Table 3). In our experience, compliance with these diets is high in this patient group. None of the children in either group were on non-steroidal anti-inflammatory drugs, corticosteroids or other immune-modulatory drugs. Other than the irregular use of approved paediatric vitamin supplements, children were not taking any non-prescription or alternative medications.

Investigations

Medical and developmental histories were taken and a routine general physical examination was performed. Serology for coeliac disease (anti-endomysial antibodies) was examined. Stool culture and microscopy, and routine serology were performed to screen for common gut pathogens.

Endoscopy

All children underwent ileo-colonoscopy under sedation or general anaesthesia. Seventy-four (50%) ASD children and 23 (77%) controls also underwent upper gastrointestinal endoscopy (Table 1). The terminal ileum was successfully visualized and biopsied in 144 (97%) ASD children and 27 (90%) controls. Ileal LNH was defined and graded as:

absent (grade 0), mild, moderate and severe (grades I–III, respectively), as described previously [2,3]. Grades were agreed by the three paediatric gastroenterologists performing the procedures. For reference purposes, images of respective grades were posted in the endoscopy room. Colonic LNH was diagnosed if hyperplastic follicles were present anywhere in the colorectum.

During ileo-colonoscopy tissue biopsies were taken from the terminal ileum and up to five colonic sites including the caecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. Biopsies from the oesophagus, stomach (antrum and/or body) and duodenum were taken during upper gastrointestinal endoscopy.

All biopsies were immersion fixed overnight in 10% formal–saline, processed into paraffin blocks and cut into 3 µm paraffin sections for histological analysis of haematoxylin & eosin stained sections.

Histopathology

Biopsies underwent routine histological reporting by the on-call surgical histopathologist and patients were treated according to the routine report. In order to standardize the histological findings in both groups, all tissue sections were then examined by a single histopathologist (A.A.) who assessed and scored each biopsy prospectively, according to a modified research proforma (Table 4), previously described and validated for the pathological changes seen in ASD children [2]. For the purpose of this study, the analysis focused upon the presence or absence of enlarged lymphoid follicles (score 0 for normal or 1 for enlarged hyperplastic follicles) in the ileum, colorectum and upper gastrointestinal tract. In addition, for each biopsy the following changes were recorded and scored as shown in Table 4. This assessment was used for the purpose of comparing an open and blinded analysis of histological sections from a sample of ASD and control patients. For this, sections from 13 ASD children (12 male, median = 7 years, range 1–11 years) and 11 controls (10 male, median = 7 years, range 1–11 years) were submitted for open and blinded histological evaluation. The two groups were of the same age and gender and underwent endoscopic assessment during the same period between 1999 and 2002. The

Table 4 Grading of inflammation

Histological finding	Histological grade, and score			
	Normal (score=0)	Mild (score=1)	Moderate (score=2)	Severe (score=3)
Acute inflammation	No interstitial neutrophils in lamina propria (LP)	Interstitial neutrophils in LP	Cryptitis	Crypt abscesses
Chronic inflammation	No increase in LP mononuclear cells	Mild increase with loss of stratification of cell density within LP	Moderate increase	Severe increase
Epithelial/LP changes	Normal	Disruption of epithelial basal lamina Condensation of LP	Erosion	Ulceration
Lymphoid follicles	Normal	Reactive changes: prominent germinal centres; tingible body macrophages	Follicular enlargement with confluence	Aphthoid ulceration
Crypts	Normal	Bifid glands Goblet cell depletion	Glandular disruption Paneth cell metaplasia	Dysplasia

controls were cases known to have neither IBD nor coeliac disease. Both assessments used the same proforma as that described above and the patient details on histological slides were masked and the slides coded by an independent observer. After this second evaluation the code was broken and the respective scores were compared. Otherwise, the anatomical site for each case was assessed for the presence or absence of any acute or chronic inflammatory changes.

Statistical analysis

The analyses were performed using SPSS 7.5 for Windows and EpiInfo 6.04b. The chi-squared test (with Yates' correction) was used to identify differences in endoscopic and histopathological features between the affected children and the control groups. The Spearman rank correlation was used to investigate the relationship between endoscopic findings and age. Wilcoxon's signed rank test and the Mann-Whitney *U* test were used in the analysis of blinded and non-blinded histological assessments. A *P* value less than 0.05 was considered to represent a significant difference.

Results

There is no significant difference between ASD and control children with respect to age and sex, with both groups being predominantly male (86% and 83%, respectively) (Table 1). None of the children had serological or histological evidence of coeliac disease. *Giardia* cysts were identified in one ASD child only and in two other ASD children, pinworms were identified in the proximal colon at ileo-colonoscopy.

Ileo-colonoscopy

A summary of macroscopic findings at ileo-colonoscopy is provided in Table 5. The most consistent and striking finding in ASD children (in whom the terminal ileum was visualized) was ileal LNH, which was detected in 129 out of 144 (90%) ASD children and in eight out of 27 (30%) controls (OR 20.42 (6.92–62.29); *P* < 0.0001).

Moderate or severe grades of LNH were present in 68% of ASD children and 15% of controls (OR 12.25

(3.82–50.77); *P* < 0.0001). These grades were combined for the purpose of statistical analysis since severe ileal LNH was not seen in the control group. In several ASD children with severe (grade III) LNH, the enlargement was so exuberant that the lumen was occluded by up to 50%.

The controls were subdivided into those with and without colonic inflammation. When these two subsets were compared independently with all ASD children, the differences are statistically significant for each (with colonic inflammation: OR 3.45 (12.9–49.43); *P* < 0.0001; without colonic inflammation: OR 51.6 (9.66–492.82); *P* < 0.0001).

Colonic LNH is significantly more common in ASD children compared with controls (OR 4.82 (1.82–13.27); *P* < 0.001) (Table 5). The distribution of enlarged follicles was often patchy and the proximal and distal colon was equally affected.

When controls were subdivided into those with and without colonic inflammation, colonic LNH was present in 31% of children with colitis and 14% of those without colitis (*P* = 0.3). When each control subgroup was compared independently, with ASD children, the prevalence of colonic LNH is significantly greater in ASD children compared with each group (with colonic inflammation: OR 3.23 (0.97–12.38); *P* = 0.03; without colonic inflammation: OR 8.8 (1.84–82.76); *P* = 0.001). Colonic LNH in ASD children was more prevalent in those with either acute or chronic colonic inflammatory changes (68/88, 77%), compared with those without such inflammatory changes (20/88, 23%) (OR 11.56 (5.41–25.04); *P* < 0.0001).

In one third of the ASD children with colonic LNH, an erythematous halo surrounded a pale follicle centre, referred to as a 'red halo' sign [21]. Other endoscopic changes, that are significantly more prevalent in the ASD group than in either of the control subgroups, include patchy loss of vascular pattern and mucosal granularity (OR 5.02 (1.43–26.88); *P* < 0.005; and

Table 5 Endoscopic findings in the ileum and colon

Finding	ASD children (n=148)	Developmentally normal control children (n=30)	Developmentally normal control children with colonic pathology (n=16)	Developmentally normal control children with no colonic pathology (n=14)
Ileum				
Normal	15 (10)	18 (67)	9 (60)	9 (75)
No LNH	15 (10)	19 (70)	9 (60)	10 (83)
Grade I LNH	31 (22)*	4 (15)	4 (27)	0 (0)
Grade II LNH	47 (33)*	4 (15)	2 (13)	2 (17)
Grade III LNH	51 (35)	0 (0)	0 (0)	0 (0)
Erythema	4 (3)*	1 (4)	0 (0)	1 (8)
Colon				
Normal	20 (14)	14 (47)	8 (50)	6 (43)
Colonic LNH	88 (59)	7 (23)	5 (31)	2 (14)
Erythema/inflammation	35 (24)*	5 (17)	2 (13)	3 (21)
Aphthoid ulceration	15 (10)*	2 (7)	1 (6)	1 (7)
Ulceration	2 (1)*	0 (0)	0 (0)	0 (0)
Loss of vascular pattern	53 (36)	3 (10)	2 (13)	1 (7)
Mucosal granularity	32 (22)	1 (3)	1 (0)	0 (0)

n (%) = number of cases (%). i = the ileum was not intubated in four of the ASD children and in three controls (one of these three had, and two did not have, colonic pathology).

*Denotes lack of statistically significant difference between ASD children and developmentally normal control children.

OR 8.00 (1.22–336.8); $P = 0.019$, respectively) (Table 5). Although colonic mucosal ulceration and erythema were also identified in both groups the differences are not significant (Table 5).

In ASD children, grade III ileal LNH is significantly more prevalent in those with colonic LNH compared with those without colonic LNH (OR 12.96 (4.36–40.08); $P < 0.0001$).

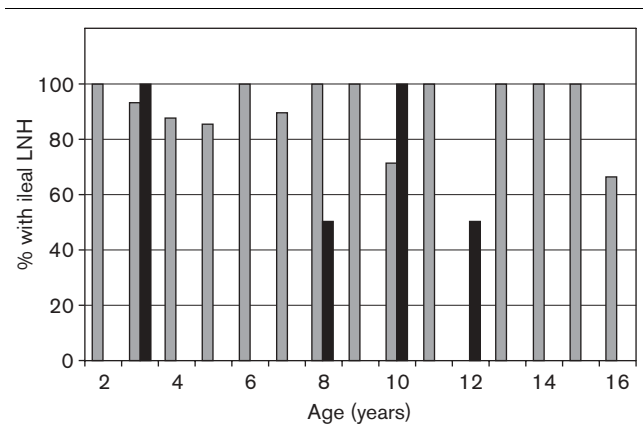
LNH was rarely seen in the absence of inflammation elsewhere in the gastrointestinal tract. Of the ASD children, two (one with grade I ileal LNH, and one with grade II ileal LNH) showed no other endoscopic or histological abnormalities. Of the controls, two (without colonic inflammation) showed no other gastrointestinal abnormalities. Overall, excluding the controls with colonic inflammation (since they were selected on the basis of having inflammation), isolated ileal LNH was detected in four of 157 children, i.e. less than 3%.

Age at colonoscopy

There was no correlation between age at ileo-colonoscopy and the presence and severity of ileal LNH (Figs 1 and 2), or the presence of colonic LNH ($P > 0.1$) in the ASD group.

Symptoms

The most common presenting symptoms in the ASD children, according to the clinical records, were constipation (75/148, 51%), diarrhoea/loose stool (45/148, 30%) and alternating constipation/diarrhoea (19/148, 13%) (Table 2). However, the overall assessment of symptoms was complex, with diarrhoea, in many cases, having an insidious onset with abdominal X-ray suggesting faecal overloading. Support for this is that, in many affected

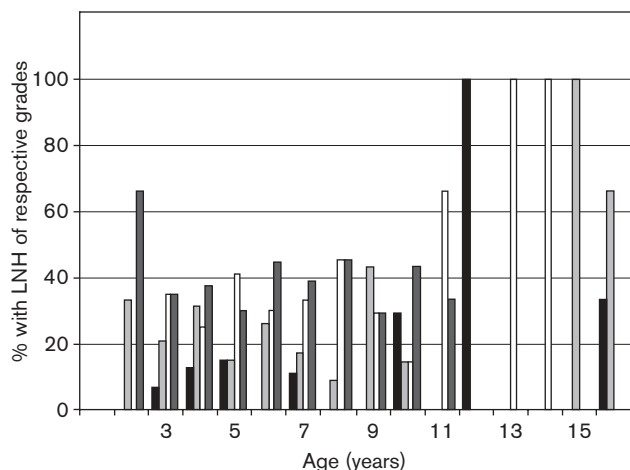
Fig. 1

Percentage of ASD children (■) and developmentally normal control children (□) with ileal LNH of any grade (I–III) presented by age at colonoscopy. There is no statistically significant relationship between presence of LNH and age ($P > 0.05$).

children, diarrhoea improved after abdominal washout and laxative drugs such as liquid paraffin. Assessment of pain as a presenting symptom is likely to underestimate, substantially, the true frequency of this symptom in ASD children due to difficulties in communication. The 30 control children were investigated mainly for abdominal pain (47%) and diarrhoea (27%).

When the ASD children in whom the ileum was visualized were divided into those that did and did not have constipation, constipation was associated with a significantly lower prevalence of severe ileal LNH (23 out of 89 with constipation and 25 out of 48 without constipation (OR 0.31 (0.14–0.71); $P < 0.002$) but an equal prevalence of colonic LNH. The prevalence and

Fig. 2



Percentage of ASD children with respective grades of ileal LNH: 0 (absent, ■), I (mild, ▨), II (moderate, □) and III (severe, ▩). There is no statistically significant relationship between grade of LNH and age ($P > 0.05$).

severity of ileal and colonic LNH in ASD children was not different between those with, and those without diarrhoea or other gastrointestinal symptoms ($P > 0.05$).

Diet

Sixty eight of 137 (50%) ASD children with a recorded dietary history were on a normal diet at the time of endoscopy. Details of dietary restrictions are provided in Table 3. One ASD child was noted to have pica but was otherwise on a normal diet. Two of 30 (7%) controls were on restricted diets, one gluten-free, the other, dairy-free. In ASD children, any dietary restriction did not affect the prevalence of ileal LNH. Colonic LNH was more prevalent in those children with any dietary restriction. However, colonic LNH was not significantly different between those on a gluten free diet, a casein free diet, or a gluten free and casein free diet.

Histopathology

Terminal ileum

A total of 161 terminal ileal biopsies were examined, including 138 from ASD children and 23 from controls. Follicular hyperplasia was identified in 84 of 138 (61%) biopsies from ASD children and two of 23 (9%) control biopsies (OR 16.33 (3.69–147.1); $P < 0.0001$). Hyperplastic follicles were enlarged with expansion of the T cell zone and a prominent germinal centre containing Tingible body macrophages and were most often multiple and confluent, with up to three large follicles being present in a single biopsy. Of the 129 ASD children who showed any endoscopic ileal LNH, 79 (61%) showed histological evidence of follicular hyperplasia. Microscopic examination of biopsy tissue underestimated the

prevalence of ileal LNH by approximately 40%. In five ASD cases follicular hyperplasia was identified by microscopy that was not reported as ileal LNH macroscopically.

Acute ileal inflammation was observed in 19 of 138 (14%) ASD children and 2 of 23 (9%) controls ($P > 0.05$). Chronic ileal inflammation was observed in 11 of 138 (8%) of ASD children and 0 of 23 controls ($P > 0.05$).

Colorectum

Histological follicular hyperplasia was diagnosed in 19 of 146 (13%) colonic biopsies from ASD children and 5 of 30 (17%) controls ($P > 0.05$). As in terminal ileal biopsies, reactive follicles were enlarged and had a prominent germinal centre containing Tingible body macrophages. There was also expansion of the T cell zone but, in contrast with the ileum, follicles were usually single and if other follicles were identified within the same biopsy the two follicles were separated by a region of mucosa devoid of any lymphoid tissue. Of the 88 ASD children showing endoscopic evidence of colonic LNH, 30 (34%) also showed histological evidence of follicular hyperplasia.

Acute inflammation of the colonic mucosa was identified in 55 of 146 (38%) ASD children and 8 of 30 (27%) controls ($P > 0.05$). Chronic inflammation of the colonic mucosa was identified in 108 of 146 (74%) ASD children but in only 4 of 30 (13%) controls; this difference is statistically significant (OR 18.47 (5.78–76); $P < 0.0001$).

In summary, in those children undergoing full ileo-colonoscopy, macroscopic and histological evaluation identified ileal LNH in 134 of 144 (93%) ASD children compared with 8 of 27 (30%) controls. Ileal LNH in the absence of mucosal inflammation somewhere in the lower gastrointestinal tract was a rare finding.

Upper gastrointestinal endoscopy

The most common endoscopic finding was erythema that was seen in the oesophagus (31%), stomach (43%) and duodenum (5%) of ASD children, and in 9%, 22% and 0% of the controls, respectively. These differences are not statistically significant. LNH was identified in the oesophagus of 2 (3%) ASD children but at no other site. There was no evidence of mucosal villous shortening in either group.

Upper gastrointestinal histology

Follicular hyperplasia was identified in the duodenum of only one ASD child but in none of the controls ($P > 0.05$). Although mild acute (1%, 4% and 6%) and chronic (17%, 19% and 15%) inflammatory changes were identified in oesophageal, gastric and duodenal biopsies respectively in the ASD group, there are no significant differences in the prevalence of these changes between

the ASD and control groups. In two ASD children, *Helicobacter pylori* was identified in gastric biopsies showing acute inflammatory changes. *Giardia* organisms were seen in the duodenal and terminal ileal biopsies of one affected child.

No granulomas were identified in biopsies taken from the lower gastrointestinal tract in ASD or control children.

Blinded histology study

When the blinded and open-label histopathological assessments were compared, there was overall agreement between the assessments in biopsies from affected children in 11 of 14 (79%). In the remaining three ASD children, additional chronic inflammatory changes were identified in the blinded assessment of two cases and additional mild chronic changes identified in the un-blinded assessment in one case. In biopsies from controls that were assessed in this way, there was overall agreement in nine of 11 cases. In the other two control cases, additional acute inflammatory changes were observed in the terminal ileum, and in the other case, additional chronic colorectal changes were identified in the blinded assessment. There is no statistically significant difference between the blinded and un-blinded histological assessments (scores) of the affected and control cases.

Discussion

This study examined the significance of ileo-colonic LNH in a large cohort of children with developmental disorders and developmentally normal controls of a similar age, both presenting with gastrointestinal symptoms. The prevalence of ileo-colonic LNH in asymptomatic children cannot be examined due to the ethical constraints of performing invasive procedures in the absence of a clinical indication. Nonetheless, this study allows certain conclusions to be drawn. Both ileal and colonic LNH are significantly more prevalent, and of greater severity, in ASD children compared with developmentally normal controls, whether or not controls have ileo-colonic inflammation. More severe grades of ileal LNH are more likely to be accompanied by colonic LNH. Ileal LNH rarely occurs in the absence of ileal and/or colonic inflammation; of a total of 178 children studied, ileal LNH was detected in the absence of associated mucosal inflammation in less than 3% of cases. The prevalence and severity of LNH is not a function of age at endoscopy.

There was discordance between the rates of endoscopic and histological detection of LNH. Histological diagnosis of follicular hyperplasia depends on adequate biopsy sampling. Follicles are not always included in a biopsy even though there was an intention to sample them. Despite this, histology identified a small number of instances of follicular hyperplasia that were not evident at

ileo-colonoscopy. Therefore, a combination of both endoscopic and histological evaluation of the mucosa provides the most comprehensive assessment of this finding and is the more accurate means of diagnosing LNH. Studies that report histological evidence of LNH only will, based upon our experience, underestimate the true prevalence of this lesion.

Endoscopic impressions of LNH are subjective, although we and others have attempted to make grading more quantitative for the purpose of systematic, prospective studies. For example, Furlano *et al.* [3] further classified mild, moderate and severe according to follicle size (grade I (follicles 2–3 mm), grade II (follicles 3–5 mm) and grade III (follicles > 5 mm)). Refinement of the definition over the course of our studies of children with developmental disorders is unlikely to account for the differences between ASD children and controls, since this refinement has applied to the evaluation of both groups, investigated contemporaneously.

A potential shortcoming of this study is that not all of the expert developmental diagnoses given to ASD children were re-evaluated in our unit. This has been performed in previous studies [1,2] that included children described herein, and we have no reason, based upon these prior observations, to doubt the accuracy of the original diagnosis based on DSM-III-R/ICD-10 criteria. All children who had a developmental disorder that was not on the autistic spectrum were excluded from further analysis, and will be the subject of a separate report. All ASD children remain under review by local developmental paediatricians and/or psychologists, and we are unaware of any case where the diagnosis has been revised.

There is a growing awareness of the high frequency of gastrointestinal symptoms in children with autism. Three prospective population surveys (a total of 1280 subjects with ASD) have been reported from Arizona [22], California [23], and the middle Atlantic region [24]. Agreement was found among the surveys that close to 20% of these children had chronic diarrhoea. Investigators will need to take into account the difficulty of obtaining a history that accurately reflects visceral symptoms, particularly pain, in autistic children. Retrospective analysis of case records will be of little value in this respect.

Any underestimate in autism will be compounded by the combination of a raised pain threshold that may be present in such children, and by their restricted ability to communicate symptoms. The situation is likely to mirror the sub-clinical 'iceberg' effect that is evident for coeliac disease [25]. Despite the shortcomings of retrospective record review, that is likely to identify only those with more severe, unambiguous gastrointestinal symptoms, Taylor *et al.* noted a statistically significant association

between documented gastrointestinal symptoms [26] and regressive autism that would be consistent with the existence of a specific phenotype.

According to the clinical records in this study, the most common presenting symptom in ASD children was constipation (51%) while in the control group the most common symptom was abdominal pain (47%). However, this should not be taken as reflecting two different symptom groups. As discussed above, accurate assessment of pain as a presenting symptom in ASD children is likely to substantially underestimate true frequency of this symptom due to difficulties in communication. We have learnt that behaviours such as self-injury, sudden outbursts of anger and abnormal posturing in order to apply pressure to the abdomen, very likely reflect pain, a phenomenon reported by others also [27]. As the major symptom, diarrhoea or loose stools was approximately equal in the ASD (30%) and control (27%) groups. In terms of specific symptoms in this study, constipation was associated with a lesser frequency of severe ileal LNH. The pathological significance of this finding, if any, is uncertain but it argues against speculation that constipation, alone, is a cause of ileal LNH.

This study was facilitated by evaluation of clinical records made prior to referral, and also prospectively taking a symptom and dietary history from the parents in the clinic and cross-checking the latter on the ward. Many children with autism are on exclusion diets, particularly a gluten and casein-free diet that has been reported to significantly benefit the development of a small group of autistic children in a recent single-blind, control study [19]. The possible benefit of this diet may be due to either allergic intolerance or possible toxic effects of, for example, gliadomorphine and casemorphine [20]. Food allergy, itself, may be associated with mucosal LNH, particularly in the duodenum, a feature that was not detected in this study. If food allergy were a major factor in the pathogenesis of mucosal disease in these children, one would have anticipated a difference in, for example, the frequency and severity of LNH between those ASD children on, and not on, exclusion diets. We did not find this. In those on any exclusion diet colonic LNH was, if anything, more common than in those whose diet was unrestricted. However, this observation might be confounded by disease severity, where those with worse disease (e.g. a greater chance of colonic LNH) are also those who may be more likely to be on an exclusion diet in an effort, by parents, to ameliorate symptoms. Alternatively, exclusion diet may be independently associated with colonic LNH though a mechanism for this is, as yet, unknown.

Radioallergosorbent (RAST) assay, although of limited value, is performed as part of the routine work-up in the unit; it was not supportive of IgE mediated food allergy as

a major factor in these children. Clearly, the influence of diet would be best assessed in a withdrawal and re-challenge setting.

In a recent analysis of lymphocyte profiles in the inflamed mucosa of ASD children by flow cytometry, the excess CD3⁺CD8⁺ and CD3⁺CD8⁻ populations were not influenced by dietary restriction [28]. Kokkonen *et al.* identified a close association between LNH of the duodenum and increased densities of intraepithelial $\gamma\delta^+$ T cells in the duodenal mucosa in children with untreated food allergies [29]. In a follow-up study of food allergic children, looking at the same phenomenon in relation to ileal LNH, these authors identified a significant increase in densities of intra-epithelial $\gamma\delta^+$ T cells and elevated $\gamma\delta^+$ /CD3⁺ ratios in the ileal mucosa, especially if the LNH was associated with colitis starting at the caecum [30]. This increase was not found in subjects with typical left-sided colitis or granulomatous Crohn's disease. Neither duodenal LNH, nor increased duodenal intra-epithelial $\gamma\delta^+$ T-cell densities have been observed in our cohort of ASD children [4] and the entero-colitis seen in children with developmental disorder appears to be distinct from that seen in paediatric food intolerance. The data indicate that dietary intolerance is not the primary effector pathway for mucosal inflammation in these children, but clearly do not preclude co-morbid food allergy in some. Nonetheless, we would agree with the impression of Kokkonen and Karttunen [13], that ileal LNH is not a normal finding in children. The unambiguous label of normal can only be attached after exclusion of associated pathology, which may be subtle at first impression.

As a follow-up to this study we will be reporting a detailed systematic analysis of the mucosal histopathology in ASD children. It is hoped that the combination of the macroscopic features described here, and the subsequent histopathological analysis will form the basis of a diagnostic profile of the gastrointestinal lesion.

In order to progress our understanding of the diagnostic significance of LNH in ASD children, the question of aetiology must be addressed further. While transient ileal LNH follows self-limited paediatric infection, ileo-colonic LNH, as seen in the present children, has also been identified in primary and acquired immunodeficiency states, including hypogammaglobulinaemia and HIV infection, where it may be associated with chronic or persistent microbial infection [14–18]. While none of the ASD children had evidence of a classical primary immunodeficiency, many suffer from recurrent infections, particularly of the upper respiratory tract, eczema and adeno-tonsillar hypertrophy (unpublished data). Immune profiling reveals a significant elevation in the number and proportion of pro-inflammatory cytokine-producing CD3⁺ lymphocytes in the small and large intestinal

mucosa and peripheral blood [30]. In another study involving some of the same cohort described here, the findings of a lymphopenia and lower quartile IgG and IgA were common [31]. The majority of children assessed showed unresponsiveness for all common recall antigens on cutaneous delayed-hypersensitivity testing, in contrast with age matched controls [32].

The links between cognitive function and gastrointestinal symptoms in ASD children remain unclear, although proposed mechanisms include a toxic entero-colonic encephalopathy and autoimmunity [33]. In an open-label study, the antibiotic vancomycin, given to treat bacterial dysbiosis, induced striking cognitive responses in children with regressive autism, maintained only during the period of administration [34]. We have proposed that these features suggest an entero-colonic encephalopathy that may be analogous, in certain respects, to hepatic encephalopathy [33] and the encephalopathy that accompanies D-lactic acidosis in some patients with short bowel syndrome [33].

In conclusion, ileo-colonic LNH is more prevalent in ASD children than behaviourally normal controls. This LNH cannot be considered a normal variant since it is rarely seen in the absence of gastrointestinal pathology elsewhere, a finding that is concordant with the work of Kokkonen and Karttunen [13].

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Conflict of interest

None declared.

Authors' contributions

AJW directed these studies and was responsible for writing the paper. KL, PA and AA collated the data and conducted the analyses. AA was responsible for the histopathological assessment of biopsies.

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