

No evidence for a clear link between active intestinal inflammation and autism based on analyses of faecal calprotectin and rectal nitric oxide

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Abstract

Aim: Due to parental concern regarding the child's bowel habits and the ongoing discussion whether there might be an association between autism and intestinal inflammation, two inflammatory markers were analysed in a group of children with autism.

Methods: Twenty-four consecutive children with autism (3–13 years) of unknown aetiology were investigated with respect to faecal calprotectin and rectal nitric oxide (NO).

Results: One child who previously had a severe *Clostridium difficile* infection displayed raised levels of both these inflammatory markers and one child with extreme constipation for whom only calprotectin was possible to measure had raised levels. The remaining children displayed results that did not indicate an active inflammatory status in the intestine when the two inflammatory markers were combined.

Conclusion: By the use of two independent markers of inflammatory reactions in the gut, i.e. rectal NO and faecal calprotectin we were not able to disclose evidence of a link between the autistic disorder and active intestinal inflammation.

INTRODUCTION

Autism is a pervasive, developmental disorder of the immature brain with multiple aetiologies (1,2). The age at onset of symptoms varies and one subgroup is referred to as early regressive (3,4), meaning that an obviously healthy child at an age of about 18–24 months loses speech and language abilities and deteriorates with respect to development and behaviour. Although several medical disorders are known to be connected with autism, the underlying cause in the individual child is often unclear (5). An association between the regressive type of autism and inflammatory, intestinal mucosal pathology has been proposed (6,7). By immunohistochemical methods Furlano et al. (8) demonstrated an increased colonic infiltration of T cells and plasma cells in children with autism and gastrointestinal symptoms, supporting that immunopathology/autoimmunity should have priority in further research. The leaky-gut hypothesis, i.e. peptides, acting as opioids, entering the nervous system, has been discussed by Shattock (9) and resulted in recommendations to exclude gluten and/or casein from the diet in children with autism. Bushara (10) discussed neurological presentation of coeliac disease and concludes that a direct association between celiac disease and autism is lacking. Findings of gastrointestinal abnormalities and symptoms, such as gastrooesophageal reflux, gastritis and disaccharide malabsorption in children with autism were reported by Horvatah et al. (11). However, research evidence are not conclusive. Whether the prevalence of gastrointestinal disorders in children with autism is increased, compared to children in general has

been studied and debated. Kuddo and Nelson (12) reviewed the literature and found no evidence upon which to base a confident conclusion as to whether gastrointestinal problems are more common in children with autism compared to children without autism. They conclude that the frequency of gastrointestinal symptoms observed in population-based samples of children with autism indicate that gastrointestinal problems are not nearly as common in children with autism as reports from paediatric gastroenterology clinics suggest. Another comprehensive review on several aspects of gastrointestinal factors in children with autism is published by Erickson et al. (13). The authors conclude that there is a lack of rigorous data to support increased gastrointestinal problems in children with autism and that available evidence indicates that standard treatment for gastrointestinal complaints in children with autism appears to be appropriate.

The proposed link between a post-vaccination status, gastrointestinal inflammation and autism has been strongly refuted by Fombonne and Chakrabarti (14) and by Taylor and collaborators (15) concluding that 'these findings provide no support for a morbilli-measles-rubella (MMR)-associated "new variant" form of autism with developmental regression and bowel problems, and further evidence against an involvement of MMR vaccine in the initiation of autism'.

Since there is still a considerable concern not only in parents but also in professionals in the field about a possible link between active gastrointestinal inflammation and autism we set out to analyse gaseous nitric oxide (NO) in the rectum as an inflammatory marker (16) and to combine this analysis with faecal calprotectin, i.e. a protein largely confined to

neutrophils (17), in children with autism to explore whether we could find indications of active intestinal inflammation in this patient group.

PATIENTS AND METHODS

Study-group

Twenty-four children (18 boys, six girls) born within the 10-year period 1991–2000, being between 3 and 13 years at the time of the study, were assessed with rectal NO and faecal calprotectin. All children fulfilled the DSM-IV criteria (18) for autistic disorder and were patients at the Neuropædiatric unit at Astrid Lindgren Children's Hospital. Children with a verified aetiology, such as syndromes with or without chromosomal aberrations were excluded. All parents presented concerns about the child's bowel habits, stools and/or worries about the discussed link between bowel inflammation and autism and wanted the child to be screened for intestinal inflammation. No child had any documented food intolerance. Gliadin/transglutaminase antibodies had been analysed on clinical grounds in 16 of the 24 children, with negative result in all. Ten of the 24 children had been referred to a paediatric gastroenterologist for assessment due to either loose stools or constipation. Two children (no. 1 and 13) had been investigated with colonoscopy, one of whom (no. 1) had a suspected ileocolonic lymphoid nodular hyperplasia. The majority, 23 children, had mental retardation (U.K. learning disabilities) in addition to autism and one child had a borderline IQ. In at least 13 of the 24 children their symptom start was described by parents and/or according to notes in the child's medical record in accordance with the early regressive type of autism.

Methods

With faecal impaction excluded, NO was measured as a primary biomarker of inflammation using a tonometer balloon catheter inserted 10 cm in the rectum and inflated with 10 mL of NO-free air. After 10 min incubation time the sample was extracted, and the NO concentration was immediately analysed with a chemiluminescence technique. The sampling technique and outcome have been described in detail (19). Reference values have previously been published elsewhere (16). Their control group comprised 23 children (mean age 9.7 years; range, 4–16 years) with low concentrations of NO, 121 ± 32 ppb, in the rectum.

Faecal calprotectin was utilized as secondary biomarker of inflammation, collected from rectal sampling at home within 7 days of NO measurements. Calprotectin was analysed using a quantitative enzyme-linked immunosorbent assay (Calprest, Eurospital SpA, Trieste, Italy). Calprotectin is a calcium-binding protein, abundant predominantly in neutrophils and it constitutes approximately 60% of their cytosolic protein. Elevated faecal calprotectin levels are observed in patients with inflammatory bowel disease (IBD). It is a non-invasive marker with high sensitivity and specificity for gastrointestinal inflammation. Reference values were obtained from a standard of 117 healthy children between 4 and 17 years (20). In their study the overall median faecal calpro-

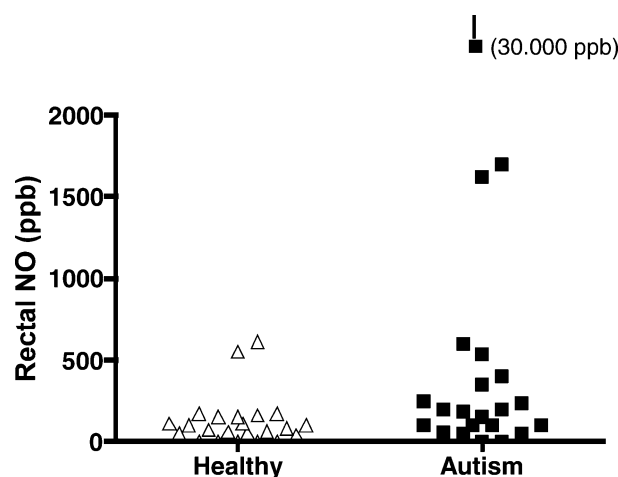


Figure 1 Grouped samples of rectal NO in healthy children and children with autism.

tectin concentration was 13.6 (95% confidence interval 9.9–19.5) $\mu\text{g/g}$ in the 17 children. In the different age groups, 4–6, 7–10, 11–14 and 15–17 years, the median calprotectin levels were 28.2, 13.5, 9.9 and 14.6 $\mu\text{g/g}$, respectively.

In cases where increased calprotectin levels were occasionally found and mismatching NO analysis a repeat test was carried out within 3 months to justify values.

The study was approved by the Ethics committee at Karolinska Hospital, Stockholm. All parents gave informed consent to let their child participate in the study.

RESULTS

NO could be analysed in 22 of the 24 patients with autism (two children could not cooperate) and calprotectin was analysed in all. Among the 22 children analysed both with NO and calprotectin, one child who had suffered from repeated *Clostridium difficile* infections at the age of 1 year had pathological values both with respect to NO and calprotectin. One child with extreme constipation, with spontaneous defecation only every 6 weeks and who could not cooperate in the NO investigation had raised calprotectin levels. When the NO and repeated calprotectin analyses were compiled in the remaining 21 children, results disclosed raised calprotectin in three children (two with normal NO, one with high NO), and a borderline value in one child (with high NO). In summary, three out of the 24 children studied had raised levels of either inflammatory marker. The distribution of values of rectal NO was similar among healthy and autistic children (Fig. 1). For further details also see Table S1. A positive correlation was demonstrated between NO and calprotectin (Fig. 2).

DISCUSSION

By the use of two separate biomarkers of an inflammatory gut reaction we have scrutinized the possibility of an active intestinal inflammatory condition in the gut of children with autism. However, the majority of the investigated

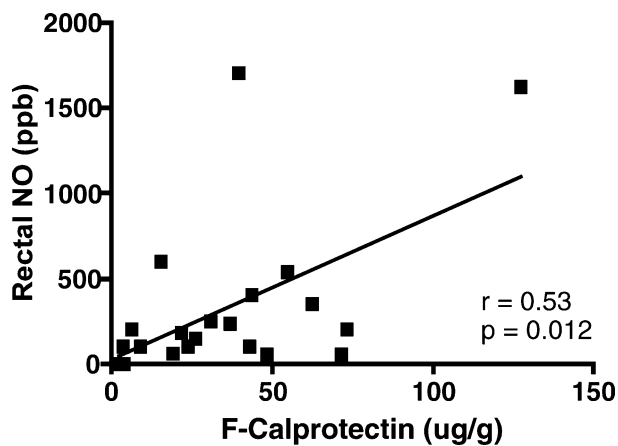


Figure 2 Correlation between rectal NO and faecal calprotectin in children with autism. For case 1 we refer to Table S1 and Figure 1.

children (19/22) turned out to display normal values when results of the two inflammatory markers were combined. In six, increased NO levels were found, almost invariably in combination with loose stools, even when calprotectin was normal. This is in line with our experience that rectal NO is correlated to an increased stool frequency (21). As a basis for our conclusion we had reference values of NO as well as calprotectin in healthy children.

There are some points that have to be taken into consideration as regards our conclusions. First, the group of patients with autism studied may have been too small and should involve more patients. The possibility of cases with inflammatory conditions in the gastrointestinal tract may therefore have been overlooked. However, the use of two different biomarkers further stabilizes our biological measurements and reduces the risk of shortcomings in our analysis. Another point is that only very few patients had been investigated by colonoscopy. However, this patient group carries the requirement of anaesthesia for that kind of investigation. Hence, in order to study a large patient group of disabled children it is preferable with screening methods based on analyse of blood, urine and faecal samples. In this context the use of faecal calprotectin seems to be favourable as no invasive step has to be taken to obtain material for analysis.

Taken together, we conclude that an intestinal inflammatory reaction does not seem to be a common feature of the autistic profile, a conclusion that is in agreement with others (12,13). Moreover, an intestinal inflammatory condition in these patients seems unlikely as they generally do not present with any signs of malnutrition or growth retardation. However, the finding that four out of 24 patients with autism had raised faecal calprotectin (in three verified by raised NO) should be interpreted with caution. When comparing calprotectin and NO as biomarkers of inflammation in the gut, the passage of luminal gas (NO) takes place within minutes whereas faecal matter (including the calprotectin protein) may get stuck inside the bowel for days until finally evacuated. This has to be taken into account when determining the temporal relationship between the two, especially in con-

stipated children. We therefore consider NO a more direct marker of on-going inflammation than calprotectin. Furthermore, one of the major physiological actions of the colon is to absorb water from the luminal contents. It is an everyday experience that more water is absorbed from faecal matter in constipated individuals, this implies that the calprotectin concentrations may be falsely high in the constipated. This is in accordance with observations reported by Bremner and collaborators (22). Among our children with autism, 10 were constipated, half of which with high calprotectin, but only two with simultaneously high NO. Hence, the yield of inflammatory reactions in the gut is considered to be lower than indicated by calprotectin alone. We therefore advocate rectal NO to be used as a feasible method for detection of inflammation in the gut. In the context of our research it seems that colonic inflammation is limited to few patients and not a global finding in autism.

A correlation between faecal calprotectin and neutrophil infiltration, i.e. acute colitis, in children with autism and gastrointestinal symptoms was demonstrated by the London group (23), concluding that faecal calprotectin is a marker for acute colitis. Such an association was not possible to detect in our study since only two children had been investigated with colonoscopy.

As calprotectin is a neutrophil-derived protein this seems to be the marker of choice to use for the detection of a gastrointestinal, on-going inflammatory disorder in individuals with autism and/or mental retardation. Earlier investigations have also proved calprotectin to be a very sensitive marker of disease (24).

To conclude, by the use of two independent markers of inflammatory reactions in the gut, i.e. rectal NO and faecal calprotectin, we found that the majority of our patients with autism did not disclose evidence of intestinal inflammation. However, a low-grade, immunopathological inflammation of the mucosa cannot be ruled out from the present study. Such a blunt inflammation has been suggested to elaborate cytokines, especially TNF α and IFN γ (25,26). One way to further improve our investigation would be to confirm the results by measuring faecal output of cytokines.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Inflammatory markers in 24 children with autism

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1651-2227.2007.00298.x>

(This link will take you to the article abstract).

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