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# Gastrointestinal Phenotype of ATR-X Syndrome

**Giuseppe Martucciello,[1] Laura Lombardi,[1] Salvatore Savasta,[1] and Richard J. Gibbons[2]***

[1]Scientific Institute (IRCCS) Policlinico "San Matteo," University of Genoa, Pavia, Italy

[2]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

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X-linked alpha thalassemia mental retardation (ATR-X) syndrome is associated with profound developmental delay, facial dysmorphism, genital abnormalities, and alpha thalassemia. Patients with ATR-X syndrome frequently present with gastrointestinal problems, in particular feeding difficulties, regurgitation and vomiting, abdominal pain, distension, and chronic constipation. Parental reports of prolonged food refusal and distress in these children are common and although these episodes are suspected to be gastro-intestinal in origin they are rarely investigated. Death in early childhood from aspiration of vomitus or from pneumonia presumed to be secondary to aspiration has been recorded in a number of ATR-X cases. In this report we review the gastrointestinal phenotype of ATR-X syndrome in 128 cases. We also demonstrate that in two siblings, regurgitation was secondary to gastric pseudo-volvulus, a condition in which the stomach does not have a normal system of peritoneal ligaments and changes position with possible torsion around itself. Furthermore, ultra-short Hirschsprung disease with colonic hypoganglionosis was shown and this may contribute to the severe constipation affecting these children.


**Key words:** ATR-X syndrome; gastro-esophageal reflux (GER); gastric pseudo-volvulus; enteric nervous system (ENS); Hirschsprung disease (HD)

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## INTRODUCTION

X-linked alpha thalassemia mental retardation (ATR-X) syndrome is associated with profound developmental delay, facial dysmorphism, genital abnormalities and alpha thalassemia [Gibbons and Higgs, 2000]. At birth generalized hypotonia is evident and in early childhood, all developmental milestones are delayed and most affected children have no speech. The facial appearance is characteristic and in most cases its recognition leads to the diagnosis. Individuals with ATR-X syndrome frequently present with gastrointestinal problems and feeding difficulties, regurgitation and vomiting, abdominal pain, distension, and chronic constipation have been reported [Gibbons et al., 1995a]. Death in early childhood from aspiration of vomitus or from pneumonia presumed to be secondary to aspiration has been recorded in a number of ATR-X cases [Gibbons et al., 1995a]. It is clear therefore, that the gastrointestinal problems in ATR-X lead to significant morbidity and mortality and in this study an attempt is made to understand the pathophysiology of the condition.

Regurgitation or vomiting and other symptoms related to feeding are typical manifestations of gastro-esophageal reflux (GER) [Mahajan et al., 1998; DeMeester et al., 1999; Orenstein, 1999; Floch, 2000]. Primary gastro-esophageal reflux refers to an incompetent lower esophageal sphincter (LES) that leads to the reflux of gastric contents into the esophagus. Secondary GER refers to a case in which an underlying condition predisposes to GER, because the stomach cannot easily empty. Examples include congenital Ladd bands, gastric pseudo-volvulus or volvulus and others causes of gastric outlet obstruction. In our cases GER symptoms were secondary to gastric pseudo-volvulus a condition in which the stomach does not have a normal system of peritoneal ligaments and changes position with possible torsion around itself [Honna et al., 1990; Darani et al., 2005].

This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html.

*Correspondence to: Dr. Richard J. Gibbons, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DS, UK. E-mail: richard.gibbons@imm.ox.ac.uk

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The differential diagnosis of constipation in childhood can be extensive and, especially for chronic constipation, it may include intestinal dysganglionoses (ID) [Meier-Ruge and Bruder, 2005]. These represent a heterogeneous group of anomalies of the enteric nervous system (ENS) including Hirschsprung disease (HD), ultrashort Hirschsprung disease (UHD), intestinal neuronal dyspalsia (IND), internal anal sphincter neurogenic achalasia (IASNA) and hypoganglionosis. These disorders have to be investigated using enzymo-histochemical protocols [Martucciello et al., 2005; Meier-Ruge and Bruder, 2005].

In this study, we analyze the associated pathology in a pair of unrelated twins affected by ATR-X syndrome and review the gastrointestinal phenotype in a large cohort of individuals with this condition.

## MATERIALS AND METHODS

### Case Studies

Initial studies consisted of barium study of the upper gastro-intestinal tract, 24-hr pH monitoring [Mahajan et al., 1998] and rectal suction biopsies with Solo-RBT in order to identify possible ID [Pini Prato et al., 2001]. Full thickness colonic biopsies were obtained laparoscopically. All biopsies were examined enzymo-histochemically for non-specific esterase and Acetylcholinesterase (*Acetylcholinesterase* reaction, BIO-OPTICA, Milan, www.bio-optica.it) [Martucciello et al., 1988, 2005; Orenstein et al., 1999; Meier-Ruge and Bruder, 2005].

### Review of Gastro-Intestinal Phenotype

This project was reviewed and approved by the United Kingdom, South East Multi-Centre Research Ethics Committee (ref: MREC02/01/02). A diagnosis of ATR-X syndrome was confirmed in 194 individuals by identifying a pathological mutation in the *ATRX* gene and/or by demonstrating the presence of HbH inclusions in red blood cells after staining with 1% brilliant cresyl blue. Clinical details concerning gastro-intestinal (GI) problems were available in 128 cases (see the online Table I at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html). One hundred four cases had been referred for diagnosis to the MRC Molecular Haematology Unit (34 of which have been previously published). In this group, referring physicians have been contacted for details regarding GI problems and in these cases radiological investigations, endoscopic findings, surgical interventions and causes of death have been obtained from hospital notes. In 42 of these cases, personally seen by RJG, this data has been supplemented by interviews with parents. In 24 cases, published by other groups, details have been garnered from the case reports. Instances of drooling, GE reflux and severe constipation were recorded as were treatment by fundoplication or gastrostomy. For the purpose of this review no distinction was made between regurgitation, vomiting and GE reflux.

## RESULTS

### Clinical Cases

Two 3-year-old non-identical twins (Fig. 1A,B) suspected to have ATR-X syndrome, had the diagnosis confirmed by observing the presence of Hemoglobin H inclusions in their red blood cells after staining with 1% brilliant cresyl blue. Deoxyribonucleic acid sequence analysis showed that they both carried the known pathological mutation 736C>T, R246C. Both children exhibited gastrointestinal problems associated with this condition: severe regurgitation of food and vomiting, dysphagia, irritability, respiratory disorders, meteorism, and chronic constipation. In addition, each presented with bilateral undescended testes and hypoplasic scrotum. In both individuals, symptoms of GER and chronic constipation were resistant to therapy.

Barium studies in both twins showed gastric pseudo-volvulus (Fig. 2A,B). Twenty-four hour pH monitoring showed severe GER with significant relationship between periods of reflux and the patients' signs (data not shown). The histochemical evaluation for AChE of rectal biopsies showed a slight increase in AChE fibers in the lamina propria mucosae and muscolaris mucosae with absence of submucosal ganglia of Meissner plexus in the last 4 cm of distal rectum. The pre-operative AChE diagnosis was that of ultrashort Hirschsprung's disease (see the online Fig. 3 at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html).

With the parents' agreement surgical treatment was undertaken. Both twins underwent laparoscopic surgery to perform trans-umbilical full thickness colonic biopsies of sigmoid, transverse, and ascending colon. Incidentally, the gonads were identified at the internal ring of inguinal canal in both patients. Nissen fundoplication and anterior gastropexy (fixation of the gastric corpus to the anterior abdominal wall) [Johnson, 1986; Honna et al., 1990; Darani et al., 2005] were performed. Anorectal dilation of the internal sphincter was performed under anesthesia at the same time, as a first procedure to treat chronic constipation.

Enzymo-histochemical studies of full-thickness colonic biopsies showed areas of hypoganglionosis of the sigmoid colon and confirmed the presence of a complex dysganglionosis: ultrashort Hirschsprung disease associated with hypoganglionosis (see the online Fig. 4 at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html).

The twins were discharged in good health, with regular oral feeding, with no GER symptoms and no

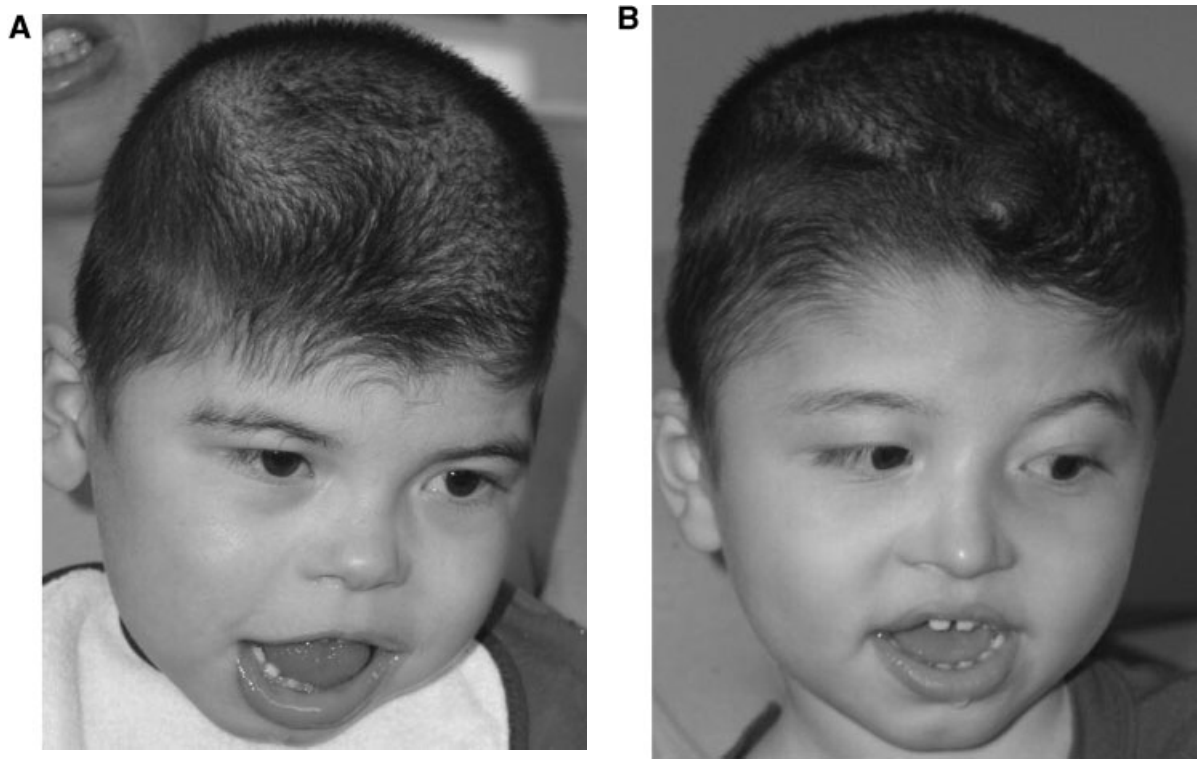


FIG. 1. **A**: Twin 1. Note the upswept hairline, epicanthic folds, upturned nose and down turned mouth which are characteristic of ATR-X syndrome. **B**: Twin 2. Note the upswept hairline, flat nasal bridge, epicanthic folds, down turned mouth and widely spaced incisors.

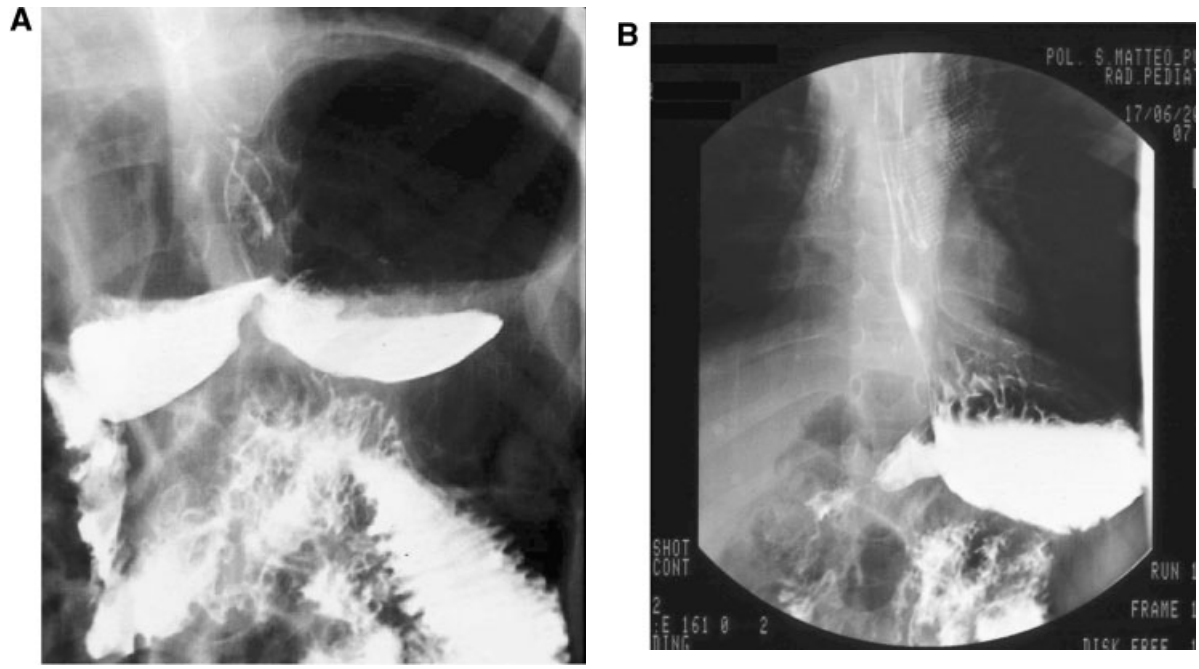


FIG. 2. **A**: Twin 1. **B**: Twin 2. In each case, the barium study of the upper gastro-intestinal tract shows gastric pseudo-volvulus with the stomach lying horizontally, the greater curvature lying in front of the lower esophagus and the pylorus facing downward.

regurgitation or vomiting. After dilatation of the internal anal sphincter we decided, with the parents' agreement, on conservative management of the constipation prior to possible surgical treatment which would involve colonic resection and endorectal pull-through (Soave procedure) [Soave, 1985] after a minimum follow-up of 6 months.

### Review of Gastrointestinal Phenotype in ATR-X Syndrome

Clinical details of GI problems were gathered in a semi-systematic manner and were available in 128 confirmed cases of ATR-X syndrome (see the online Table I at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html). Drooling was reported in 36% of cases; GE reflux was present in 72% and constipation in 30%. Ten percent of cases were treated by fundoplication and 9% were fed by gastrostomy. Upper GI bleed was reported in 10% of cases. Three cases died following aspiration of vomitus. Malrotation of the gut or volvulus was seen in four cases and in three of these this led to infaction of the gut from which two children died. In four cases there was recurrent admission to hospital for ileus or pseudo-obstruction. Furthermore we received many anecdotal reports from parents describing prolonged episodes of patient distress with refusal to eat or drink some of which required hospital admission for rehydration but in which the cause was not determined.

## DISCUSSION

X-linked alpha thalassemia mental retardation syndrome is an X-linked recessive condition, which results from mutations in the *ATRX* gene [Gibbons et al., 1995b]. ATRX protein is a chromatin remodeling protein and, based on the fact that the alpha thalassemia often seen in this condition results from down regulation of alpha globin expression, it is thought that the protein's function is to facilitate gene expression. The complexity of the clinical phenotype probably reflects the repertoire of genes, which require ATRX for their expression. As yet the other target genes for ATRX are unknown. The findings in this report suggest that some of these genes are also involved in normal gastrointestinal function.

Gastrointestinal problems are very common in ATR-X syndrome. They cause significant morbidity and mortality and present a considerable management challenge. Parents frequently remark on excessive drooling, recurrent vomiting or regurgitation and constipation affecting the children with this condition. Here we report on the investigation and treatment of GE reflux and severe constipation in non-identical twins affected by ATR-X who were found to have gastric pseudo-volvulus and ultra-short Hirschsprung disease with colonic hypoganglionosis.

Gastric pseudo-volvulus is a condition in which the stomach has not a normal system of peritoneal ligaments and "fixation," so the stomach can change position with possible organo-axial or mesenteroaxial rotation [Honna et al., 1990; Darani et al., 2005]. Typically the gastric volvulus is related to the absence or laxity of the gastrocolic and gastrosplenic ligaments. These anatomic attachments fix and prevent rotation of the stomach. The gastric pseudo-volvulus may present as an acute, subacute, or chronic problem and may be underdiagnosed. Clinical findings appear to be related to the degree of rotation and subsequent gastric obstruction. The main symptoms of chronic pseudo-volvulus are nonspecific and included vomiting, post-prandial gastric distension and abdominal pain. The diagnosis is confirmed by upper gastrointestinal contrast studies. The main radiological signs included the horizontal lie of the stomach, the greater curvature lying in front of the lower esophagus and the pylorus facing downward [Honna et al., 1990; Darani et al., 2005]. The surgical procedure involves anterior gastropexy and when there is severe gastroesophageal reflux disease, Nissen fundoplication is performed during the same procedure [Johnson, 1986; Honna et al., 1990; Darani et al., 2005].

Ultrashort Hirschsprung disease (UHD) is an intestinal dysganglionosis which is either limited to the anal ring or extends a maximum of 4 cm into the distal rectum. Typically, UHD develops progressively with symptoms of chronic constipation in the second half of the first year of life. A reliable diagnosis of UHD needs an enzyme-histochemical AChE reaction in sections of rectal mucosa. In contrast to classical Hirschsprung disease, scarcely any nerve fibers with increased AChE activity are present in the lamina propria mucosae. An increase of AChE activity can be found in the muscularis mucosae and the musculus corrugator cutis ani. The therapy of choice is sphinctero-myectomy, if dilation of the internal sphincter proves ineffective. In the intestinal phenotype of these two cases of ATR-X, ultrashort Hirschsprung disease was associated with areas of myenteric hypoganglionosis. Colonic hypoganglionosis is an inborn disease characterized by low cholinergic tone in the muscularis propria and hypoplasia of the myenteric plexus [Meier-Ruge and Bruder, 2005].

In the light of the high frequency of general gastrointestinal problems in ATR-X cases we propose that every ATR-X syndrome patient should be accurately studied including AChE activity, 24-hr pH monitoring, and barium study of the upper gastro-intestinal tract.

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