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CASE REPORT

RET promoter variations in familial African degenerative leiomyopathy (ADL): first report of a possible genetic-environmental interaction

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Abstract African degenerative leiomyopathy (ADL, DL, Bantu pseudo-Hirschsprung's disease) is a distinctive visceral myopathy, of unknown etiology, occurring in Africa. It has a classical clinical and histologic picture in young indigenous African children. It presents as intestinal pseudo-obstruction with a massive megacolon due to degeneration of smooth muscle without aganglionosis. Because of its late presentation and geographical and ethnic distribution, it is thought to be an acquired degenerative hollow visceral myopathy. Only one previous report of familial recurrence exists. The main Hirschsprung susceptibility gene RET is a potential candidate gene in this condition, because of its role in the development of the intrinsic innervation and ganglia of the smooth muscle layers of the gastro-intestinal tract. We report a second case of familial ADL recurrence and explore possible etiologic causes including variations of the RET gene. Multiple variations in the RET promoter were identified in this case which leads to the possibility of a genetic-environmental predisposition for this condition. We therefore hypothesize that RET may play a modulating role in ADL susceptibility (and possibly other visceral myopathies). It is possible that subtle malformations in the ENS may result from RET dysfunction which then predisposes the individual to environmental influences which initiate the later onset of muscle degeneration.

Keywords Visceral myopathy · African degenerative leiomyopathy · ADL · RET proto-oncogene · Gene–environmental interaction

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Introduction

African degenerative leiomyopathy (ADL, DL, Bantu pseudo-Hirschsprung's disease) forms a part of the relatively rare group of complex visceral myopathies which result in a functional or pseudo-obstruction [1, 2]. It is a distinctive form of acquired degenerative visceral myopathy of uncertain etiology which occurs largely in Africa and results in progressive functional intestinal obstruction [3, 4]. It remains difficult to identify and treat with patients eventually developing malnutrition and starvation as a result of the deranged motility. The majority of patients are subjected to a protracted debilitating illness which often eventually results in death.

ADL is generally accepted as an acquired condition possibly caused by environmental toxins which promote the progressive degeneration of smooth muscle in the intestinal wall (predominantly colon) [4, 5]. It usually presents in older children, rarely occurring within the first year of life [3–7]. It is an interesting condition because, unlike known familial myopathic causes of chronic idiopathic intestinal pseudo-obstruction (CIIP) [8], ADL rarely affects other family members, this being only the second reported such case [9].

We present a case of ADL recurrence in an affected sibling, thus raising the pertinent question of a possible

genetic connection and a gene–environmental interaction. We report for the first time a connection between ADL and homozygous genetic variations within the RET promoter region and explore the possibility that both genetic and environmental factors contribute to disease pathogenesis.

Case report

An 11-year-old African female presented with an acute on chronic history of abdominal distension since 2004 (Fig. 1). There was a family history of her sister who had been treated for ADL in the same unit but having died as a result of it some years previously at the age of 9 years. None of the other seven siblings, or the parents, appeared to be affected. There was no history of allergies or herbal enema administration.

On X-ray, a massive megacolon was observed which almost filled the abdomen. The bowel was also markedly distended at laparotomy so much so that a diagnosis of a sigmoid volvulus was considered.

Biopsies taken from the splenic flexure showed a grossly abnormal muscularis propria but an intact normal mucosa and a normal submucosa. Normal ganglion cells were present and there was no increase in AChE on the Meier-Ruge staining technique. However, fibrosis of the muscularis propria was fairly marked in the longitudinal and circular muscle layers with the longitudinal layer being more affected. Degenerative features were also demonstrated within the muscle cells with hypereosinophilia of the cytoplasm, pyknotic hyperchromic nuclei and nuclear pleomorphism, as well as signs of a chronic lymphocytic infiltrate. A second specimen showed severe fibrosis of the longitudinal muscle with displacement of the Auerbach inter-myenteric plexus into the circular muscle layer. These fibrotic fibres were highlighted on Masson Trichrome staining.

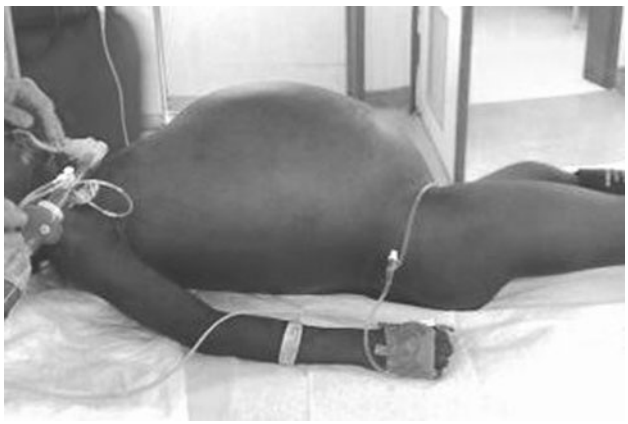

Fig. 1 Chronic abdominal distension. Patient supine in theatre

Due to the family history of having lost one child, further surgical intervention was declined by the family and the patient was therefore treated with a low residue diet and/or Neostigmine (Rae's mixture). The abdomen remains much distended and further surgery (venting stoma) may well be required.

DNA testing methods

Blood samples were forwarded to Tygerberg Hospital and the University of Stellenbosch for testing. DNA was extracted from whole blood and tissue samples by standard DNA extraction procedures [10, 11] and polymerase chain reaction (PCR) amplification (21 exons of the RET gene) was performed using previously designed intronic primers. PCR products were screened for genetic variation of the RET proto-oncogene. Successful PCR amplifications were subjected to semi-automated bi-directional sequencing analysis. DNA sequencing was performed on PCR products demonstrating mobility or conformational variants in the PAA gels, using an ABI 3100 PRISM automated sequencer (Applied Biosystems, Foster City, CA, USA).

DNA results

DNA extraction, PCR and gene testing of the RET gene promoter showed five homozygous variations, in the promoter region of the RET proto-oncogene (Table 1). The rest of the gene was normal. These are all known variants which have been previously associated with Hirschsprung's disease. The fact that they are homozygous in this case suggests some dysfunction of gene expression.

Discussion

African degenerative leiomyopathy is a distinctive non-familial myopathic degeneration which presents as an intestinal pseudo-obstruction without mechanical causes of intestinal obstruction. It appears to be region specific,

Table 1 RET promoter variations: familial ADL

RET promoter variant	rs number	Hetro/homozygous
5′UTR-1782 A/G	rs2505998	HOM
5′UTR-1697 C/G	rs3026727	HET
5′UTR-1260 C/T	rs2505999	HET
5′UTR-719 T/C	rs2435366	HOM
5′UTR-200 A/G	rs10900296	HOM
5′UTR-196 C/A	rs10900297	HOM

HOM homozygous, *HET* heterozygous

mostly affecting young Africans of Southern, Central and East Africa, although patients from other ethnic groups in North Africa have also been identified [3, 12]. The term "African degenerative leiomyopathy" is therefore probably a valid scientific description in order to distinguish it from other visceral myopathies occurring elsewhere [3].

The clinical symptoms begin after a number of years suggesting that the disease is acquired rather than being congenital in origin, possibly as the result of environmental toxins [5]. ADL begins as a visceral myopathy, initially targeting the distal large bowel and progresses proximally. Clinically, it is characterized by a long history of increasing abdominal distension due to intestinal pseudo-obstruction. Subsequent megacolon usually extends as far as the anorectal junction and may be difficult to distinguish from a sigmoid volvulus. Although it mostly occurs in the older child, with the mean age at presentation being ±9.5 years [4], an earlier presentation has been described as early as 6 months [5]. In the light of only one previous report of intra-familial clinical association [9], there has been little support to date for a genotype–phenotype correlation. This report of a familial association suggests that both genetic modifiers as well as environmental factors probably contribute to disease pathophysiology.

The primary ADL target appears to be the large bowel and presents with massive abdominal distension as the result of a functional or pseudo-obstruction [2, 4]. The condition may, however, extend proximally from the primary site in the large bowel into small bowel and in a few cases has been shown to affect the entire gastrointestinal tract [3]. The progression of the disease is clearly based on the degeneration of intestinal smooth muscle which in turn results in poor intestinal motility. This is then associated with progressive abdominal distension, megacolon and marked gaseous distention and may result in acute presentation. Malnutrition results from poor intestinal function, absorption and bacterial overgrowth. The accumulation of intra-luminal fluid and bacterial overgrowth that results from stasis probably accounts for malabsorption, subsequent malnutrition and progressive downhill course of patients [8]. Stool examination has generally failed to reveal any significant causative pathogenic organisms [5], suggesting that it is secondary to the bacterial overgrowth.

In contrast to reports of North American patients with CIIP [13], there is no primary abnormality of the myenteric plexus (apart from displacement of ganglion cells due to fibrosis), no congenital degeneration of smooth muscle or abnormal development of nerve plexii. In addition, they generally present with recurrent attacks of abdominal pain, distension and vomiting. Involvement of the urinary bladder appears to be common to most of these conditions, but is usually seen later in DL than in other forms of visceral myopathy [8]. In the case of ADL, only one previous report of familial recurrence has been published [9].

This report shows the first association between a visceral myopathy and the promoter region of the main Hirschsprung-associated gene (RET). RET is a potential candidate gene in this condition, not least due to its causative association with other intestinal motility disorders such as Hirschsprung's disease but also because of its role in the development of the intrinsic innervation and ganglia of the smooth muscle layers of the gastro-intestinal tract. The RET variants found in this study are all known to have been previously reported in association with Hirschsprung's disease. The fact that the variations are homozygous allele changes without other changes in the RET gene body suggests some disturbance of gene function. Variations in the promoter area of a gene can affect gene regulation as the recognition sites of gene enhancers and suppressors in this region may not recognize the altered site. This may then significantly impact on gene function and expression, as genetic changes in the RET proto-oncogene result in a non-functional RET protein that fails to interact with critical growth factors or transmit signals to other signaling cascades within cells.

Although the connection between this and a visceral myopathy is difficult to understand, a concept of a genetic-environmental interaction is not unknown, having been described in other myopathic conditions [14, 15]. We therefore hypothesize that RET may play a modulating role in ADL (and possibly other visceral myopathy) susceptibility. It is possible that subtle malformations in the ENS (including the autonomic nervous system) may result from RET gene dysfunction which then predispose the individual to environmental influences which initiate the onset of degeneration.

This second case of a familial recurrence therefore suggests a geno-phenotype correlation and this first report of a possible genetic association and further investigation of this association should be carried out in future cases of visceral myopathy.

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