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ORIGINAL ARTICLE

Recurrent ACTG2 gene variation in African degenerative visceral leiomyopathy

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Abstract

Introduction Visceral myopathies remain difficult and frustrating clinical entities, a distinctive form of acquired degenerative visceral myopathy, African degenerative leiomyopathy, a myogenic functional intestinal obstruction without aganglionosis which affects smooth muscle of the intestine, in young indigenous African children. The Actin G2 gene is the main gene encoding smooth muscle actin found in enteric tissues. Recent research has identified Actin G2 alpha gene variation as an important causative biomarker in visceral myopathies and megacystis microcolon. This study of the Actin G2 gene (ACTG2) in an African population explores a possible molecular basis abnormal muscle function in a visceral myopathy.

Patients and methods Following ethical permission and informed consent, DNA was extracted from whole blood samples in five patients with histologically proven African degenerative leiomyopathy. PCR amplification of ACTG2 alpha gene products by semi-automated bi-directional sequencing analysis. Results were analysed using FinchTV Sequence Alignment Software and predicting bioinformatic investigation by PolyPhen 2 software.

Results Five new patients with the ADL phenotypes were prospectively investigated for variation in the Actin G2 gamma gene (ACTG2). ACTG2 gene variation occurred in exon 5 (c.463 A>G K119R), in three (60%). In addition, intronic variation t > c-IVS3 was identified in three with the K119 mutation plus further g > c -IVS12 and t > c + IVS16(2), suggesting a possible haplotype. Bioinformatic modelling showed that these ACTG2 gene variations are highly non-conservative altering protein expression.

Conclusions Recurrent Actin G2 smooth muscle gene variation in African degenerative visceral leiomyopathy is associated with abnormal muscle actin development.

Keywords Visceral myopathy · African degenerative leiomyopathy · ADL · Children

Introduction

Visceral myopathies remain difficult and frustrating clinical entities, creating dilemmas for both gastroenterologist and Paediatric surgeons. A distinctive form of acquired degenerative visceral myopathy, African Degenerative leiomyopathy (ADL, DL, Bantu pseudo-Hirschsprung disease) is a distinctive visceral myopathy of uncertain etiology, occurring in young indigenous African children. ADL forms part of the relatively rare group of complex visceral myopathies which result in a functional or pseudo-obstruction [1, 2], remaining difficult to identify and treat. It presents with a massive megacolon and pseudo-obstruction presumably due to degeneration of smooth muscle without aganglionosis and a distorted enteric nervous system due to fibrosis.

ADL has until recently been accepted as being an acquired condition, resulting from the progressive degeneration of smooth muscle in the intestinal wall (predominantly colon) [3, 4]. Unlike known familial myopathic causes of chronic idiopathic intestinal pseudo-obstruction (CIIP) [5], DL rarely affects other family members although there are reports of familial recurrence [6, 7]. Outcome is mostly poor, with the majority of patients being subject to a protracted debilitating intestinal malfunction, with malnutrition and

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starvation frequently resulting from the deranged motility which often eventually results in death.

A visceral myopathy involves pathology of intestinal muscle. Muscles consist of actin and myosin fibres. The Actin G2 gene (ACTG2) has been identified as the main gene encoding actin gamma 2, a smooth muscle actin found in enteric tissues. Recent research has shown that alpha smooth muscle actin expression is an important biomarker and is a possible cause in other visceral myopathies including megacystis microcolon [8–13].

This study of the ACTG2 in patients with visceral myopathy in African population explores a possible molecular basis for the abnormal muscle function.

Methods

Following ethical permission and informed consent, samples of DNA, extracted from whole blood samples in five patients with histologically proven ADL, were subjected to DNA extraction, followed by PCR amplification of the ACTG2 gene. Semi-automated bi-directional sequencing analysis used FinchTV Sequence Alignment Software (http://en.biosoft.net) to read chromatogram files.

Bioinformatic modelling

Further bioinformatic in silico modelling utilized the ClustalW2 multiple sequence alignment program (to confirm species conservation) as well as Project HOPE (http://www.cmbi.ru.nl/hope) to model the significance of the nucleic acid change. The SWISS modelling software determined the protein structural changes following translation. The PolyPhen 2 software (genetics.bwh.harvard.edu/pph2/) was used to predict the significance of the amino acid change. The Human Splice Finder (Bioinformatics and Genetics Team, Aix Marseille Université) was used to predict the significance of the intronic variants.

Ethical permission

This research project has been approved by the research committee at Stellenbosch University (C019/2001) and was conducted according to the accepted ethical codes and guidelines, as outlined in the declaration of Helsinki.

Results

Five new patients with clinically and histologically confirmed ADL from the South African population were prospectively investigated for Actin G2 alpha gene variations. In all patients, the diagnosis of ADL was confirmed on full thickness rectal biopsy, which showed characteristic features of ADL. The bowel was thin walled and very dilated. Microscopy showed smooth muscle degeneration and necrosis with replacement fibrosis of muscular layers and displacement of ganglion cells; there was a mild increase in numbers and thickness of nerve fibres. There was also mild inflammatory changes and medial fibrosis of small muscular arteries. Immunohistochemical studies were within normal limits.

DNA results

The results of ACTG2 gene analysis are documented in Table 1.

Genetic variation K119R was identified in exon 5 (c.463 A>G K119R), of the ACTG2 gene in three of the five patients (60%). In addition, a t>c-IVS3 intronic variation in exon 5 was identified in the same three patients. A further g>c-IVS12 intronic variation was identified in exon 3 (one with and one without the exon 5 mutation) and a t>c+IVS16 intronic variation was identified in exon 7 in two patients who also showed the exon 5 mutation.

Bioinformatic modelling showed that the residue replacements in the variant (Lys to Glu) is highly non-conservative and variation can alter interactions within the protein conformation.

Table 1 Table of genetic variation in the ACTG2 alpha gene in patients with ADL

Sample no.	Intron	Exon3	Intron 3	Exon 4	Exon 5	Exon 6	Intron 6	Exon 7	Exon 8	Exon 9
ADL01		WT	t>c-IVS3	WT	c.463 A>G K119E	WT		WT	WT	WT
ADL02	g>c -IVS12	WT		WT	WT	WT		WT	WT	WT
ADL03	g>c -IVS12	WT		WT	WT	WT		WT	WT	WT
ADLO4	g>c -IVS12	WT	t>c-IVS3	WT	c.463 A>G K119E	WT	t>c+IVS16	WT	WT	WT
ADL05		WT	t>c-IVS3	WT	c.463 A>G K119E	WT	t>c+IVS16	WT	WT	WT

WT wild type

Discussion

Degenerative leiomyopathy (DL) appears to be a region-specific condition, mostly affecting young Africans of Southern, Central and East Africa [14, 15], although patients from other ethnic groups have also been identified. However, all appear to relate to Africa [15] and include patients from Central and North Africa as well as patients of African origin living overseas.

The term "African degenerative leiomyopathy (ADL)" is, therefore, probably a valid scientific description to distinguish it from other visceral myopathies [15]. Clinically, ADL is characterized by a long history of increasing abdominal distension due to intestinal pseudo-obstruction and it begins as a visceral myopathy, initially targeting the distal large bowel, with the megacolon usually extending to the anorectal junction. 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 presentations.

Because of the delayed onset, with the clinical symptoms beginning after a number of years (mean age at presentation being ± 9.5 years [3]), it is generally accepted that the disease is acquired rather than being congenital in origin. Reports of familial cases and other genetic associations [6, 7] suggest that both genetic and/or environmental factors contribute to disease pathophysiology.

Because ACTG2 gene has been identified in patients with megacystis microcolon and other visceral myopathies [8–13] an important role for contractile proteins in smooth muscle disease has been suggested [11]. It has long been established that smooth muscle layers of the gastro-intestinal tract consist of two organized layers of circularly or longitudinally oriented muscle bundles. These muscles are made up of fibres of actin and myosin. The specialized binding of actin and myosin results in cross-bridge formation and cycling, resulting in force generation within smooth muscles. It is important to note that the anatomy of these layers differs in smooth muscle from the sarcomeres of skeletal muscle in that the actin fibres are laid down as a network over the myosin in smooth muscle. This structure allows the unique gastrointestinal motility patterns to occur.

Although these patterns of contractile activity in gastrointestinal muscles are determined by inputs from a number of motor neurons, they do not depend wholly upon nerve signaling but rely on spontaneous electrical activity from intrinsic pacemaker activity emanating from interstitial cells of Cajal as well as the ability of the smooth muscle fibres to respond to these stimuli. Motility disorders can, therefore, result from developmental failures and processes that compromise function of gastrointestinal muscle.

The Actin gamma 2 protein is a smooth muscle actin found in enteric tissues encoded by the ACTG2 gene. Recent research has indicated that deficiencies in the ACTG2 gene play a role in the complex changes in smooth muscle-specific protein expression in a variety of conditions. Dysfunctional states such as visceral myopathies have been related to variations within the ACTG2 gene [8–10].

Our study links, for the first time, ADL to a spectrum of intestinal smooth muscle conditions through significant variations within the ACTG2 gene which encodes γ2 enteric actin in the intestinal smooth muscle. The recurrence of the c 463 A > G K119R mutation in exon 5 in 60% of patients appears significant both from bioinformatics modelling, where it is predicted on SIFT scoring (0.00.) with a median sequence conservation score of 3.15 to affect protein function. This variation has also been reported in one of 11 patients with MMIHS subjected to whole exome screening [11]. The further heterozygous noncoding variant observed on exon 3, did not appear to have any effect on the splice site,

From this study, there appears to be sufficient evidence to regard the exon 5 of the ACTG2 gene as an important target area in the pathophysiology of ADL and potentially other visceral myopathies.

The case for the significance of exon 5 variation is strengthened by the t > c-IVS3 intronic variation in exon 5 in three patients with the K119R mutation.

The additional, a g > c-IVS12 intronic variation occurs on the intronic region before exon 3 and occurs in a heterozygous manner with base substitution from g to c. This variant appears to lie on the exonic splice site and creates a splice silencer, which inhibits the splicing of introns prior to m-RNA translation. The additional t > c + IVS16 variation in intron 7 is of unknown significance. However, it is worth noting that the intron 7 patients also had the exon 5 K119R mutation and this may relate to a modifying haplotype.

Conclusions

The actin smooth muscle gene (ACTG2) showed variation in 60% of samples and is the first gene associated with abnormal functioning muscle in patients with African degenerative leiomyopathy.

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References

1. Puri P, Rolle U (2004) Variant Hirschsprung's disease. Semin Pediatr Surg 13:293–299. https://doi.org/10.1053/j.sempedsurg.2004.10.017
2. Moore SW, Schneider JW, Kaschula ROC (2002) Unusual forms of intestinal myopathy. Pediatr Surg Int 18:13–20. https://doi.org/10.1007/s003830200003
3. Rode H, Moore SW, Kaschula ROC, Brown R, Cywes S (1992) Degenerative leiomyopathy—a clinico-pathological study. Pediatr Surg Int 7:23–29
4. Moore SW, Schneider JW, Kaschula ROC (2002) Non familial visceral myopathy clinical and pathologic features of African degenerative leiomyopathy. Pediatr Surg Int 18:6–12. https://doi.org/10.1007/s003830200002
5. Schuffler MD, Lowe MC, Bill AH (1977) Studies in idiopathic intestinal pseudo obstruction. 1. Hereditary hollow visceral myopathy: clinical and pathological studies. Gastroenterol 73:327–338 **(PMID 873134)**
6. Hamilton DG, Wainwright HC, Isaacson C (1980) Pathology of hereditary hollow visceral myopathy. Proc XIIIth Int Congr Int Acad of Pathol, IAP, Paris, p 47
7. Van Rensburg C, Moore SW, Zaahl M (2012) RET promoter variations in familial African degenerative leiomyopathy (ADL): first report of a possible genetic-environmental interaction. Pediatr Surg Int 28:1235–1238 **(PM 23053599)**
8. Matera I, Rusmini M, Guo Y, Lerone M, Li J, Zhang J, Di Duca M et al (2016) Variants of the ACTG2 gene correlate with degree of severity and presence of megacystis in chronic intestinal pseudo-obstruction. EurJ Hum Genet 24:1211–1215 **(PM 26813947)**
9. Halim D, Hofstra RM, Signorile L, Verdijk RM, van der Werf CS, Sribudiani Y et al (2016) ACTG2 variants impair actin polymerization in sporadic megacystis microcolon intestinal hypoperistalsis syndrome. Hum Mol Genet 25:571–583 **(PM 26647307)**
10. Lu W, Xiao Y, Huang J, Tao Y, Yan W, Lu L, Cao Y, Cai W (2016) Mutation in actin gamma-2 responsible for megacystis microcolon intestinal hypoperistalsis syndrome in 4 Chinese patients. J Pediatr GastroenterolNutr 63:624–626 **(PM 27007401)**
11. Wangler MF, Gonzaga-Jauregui C, Gambin T, Penney S, Moss T, Chopra A et al (2014) Heterozygous de novo and inherited mutations in the smooth muscle actin (ACTG2) gene underlie megacystis-microcolon-intestinal hypoperistalsis syndrome. PLoS Genet 10:e1004258 **(PM 24676022)**
12. Milunsky A, Baldwin C, Zhang X, Primack D, Curnow A, Milunsky J (2017) Diagnosis of chronic intestinal pseudo-obstruction and megacystis by sequencing the ACTG2 gene. J Pediatr Gastroenterol Nutr 65:384–387 **(PM 28422808)**
13. Lehtonen HJ, Sipponen T, Tojkander S, Karikoski R, Jarvinen H, Laing NG et al (2012) Segregation of a missense variant in enteric smooth muscle actin gamma-2 with autosomal dominant familial visceral myopathy. Gastroenterology 143:1482–1491 **(PM 22960657)**
14. Isaacson C, Wainwright HC, Hamilton DG, Ou Tim L (1985) Hollow visceral myopathy in black South Africans—a report of 14 cases. S Afr Med J 67:1015–1017 **(PMID 3839321)**
15. Kaschula ROC, Moore SW, Rode H, Brown RA, Cywes S (1993) Degenerative leiomyopathy in African children. East Afr Med J 70:37–39
16. Knight RD, Mebus K, d'Angelo A, Yokoya K, Heanue T, Roehl H (2011) Ret signalling integrates a craniofacial muscle module during development. Development 138:2015–2024 **(PM 21490065)**