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REVIEW ARTICLE

Total colonic aganglionosis and Hirschsprung's disease: a review

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Abstract Total colonic aganglionosis is a relatively uncommon form of Hirschsprung's disease (HSCR). It occurs in approximately 2–13 % of HSCR cases and involves the entire colon which is aganglionic but may extend proximally into varying lengths of small bowel. As a result, it should be separated into Total colonic aganglionosis (TCA) [defined as aganglionosis extending from the anus to at least the ileocaecal valve but no more than 50 cm small bowel proximal to the ileocaecal valve] and total colonic and small bowel aganglionosis (TCSA) which may involve very long segments of small bowel aganglionosis. Clinically, TCA appears to represent a different spectrum of disease in terms of presentation and difficulties which may be experienced in diagnosis suggesting a different pathophysiology from the more common forms of HSCR. It is therefore not yet clear whether TCA merely represents a long form of HSCR or a different expression of the disease. A number of differences exist between TCA and other forms of HSCR which require explanation if its ubiquitous clinical features are to be understood. In addition to the usual explanations for the aganglionosis of HSCR, there is some evidence suggesting that in place of being purely congenital, it may represent certain different pathophysiologic mechanisms, some of which may continue to be active after birth. This study reviews what is known about the clinical, radiological and histopathologic differences between TCA and the more frequently encountered recto-sigmoid (or short-segment; S-HSCR) and correlates them with what is currently known about the genetic and molecular biologic background to find possible pathogenetic mechanisms.

Keywords Hirschsprung · Hirschsprungs disease · Total colonic aganglionosis (TCA) · Genetic

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Introduction

Hirschsprung's disease (HSCR) can be regarded as a collection of conditions which produce a functional intestinal obstruction and which have aganglionosis of the intermyenteric plexuses as a common feature. The aberrant colonization of the enteric nervous system (ENS) neuroblasts during development, which occurs in HSCR [1], is thought to result from disruption of normal signaling due to several genetic variations (on at least 12 genes), which determine its final phenotypic expression [2, 3].

Clinically, Hirschsprung's disease has previously been classified into ultra-short, short segment (S-HSCR) and long segment (L-HSCR) [4]. The latter can probably be divided into colonic, total colonic aganglionosis (TCA) and total colonic with small bowel aganglionosis (TCSA) which may involve a very long-segment HSCR (Zuelzers disease) [5].

Total colonic aganglionosis (TCA) is an uncommon form of HSCR occurring in approximately 2–13 % of cases [6–8]. TCA has long been recognized as presenting particular problems in diagnosis [9, 10] and management [11–14]. The incidence of TCA in a Japanese population averaged 1 in 58,496 with a male:female ratio of 1.5:1 over a 30-year period [15]. Affected families are known to carry approximately 200 times higher risk of recurrence [2], particularly (but is not confined to) in patients with long-segment aganglionosis (L-HSCR) [16–18]. TCA has been reported to recur in 15–21 % [19] and as high as 50 % in

patients with ultra-long-segment aganglionosis (TCSA) [20].

Because the presentation and special problems associated with very long aganglionic segments in TCSA, TCA has been defined as aganglionosis extending from the anus to at least the ileocaecal valve but no more than 50 cm proximal to the ileocaecal valve [21]. It is thus regarded as separate from the extended intestinal form (or TCSA) as well as the very rare form of aganglionosis which stretches from duodenum to anus [22, 23]. It is not yet completely clear whether separation of these two entities is justified in terms of pathogenesis and biology, further research being required.

TCA is generally regarded as a special problem area in the HSCR spectrum of disease. Although it does share the common feature of aganglionosis with other forms of HSCR, it differs in certain respects. For example, the expected 4:1 male predominance of short-segment aganglionosis (S-HSCR) decreases to 1:1 or even 0.8:1 in TCA [12, 17, 24]. Clinically, TCA also appears to represent a different spectrum of disease in terms of presentation and difficulties which may be experienced in diagnosis suggesting a different pathophysiology from the more common forms of HSCR. Verification of the latter could possibly explain the late presentation of a number of TCA patients who present later than anticipated considering the severity of disease. Some have even gone as far as to suggest that it be regarded as a separate condition. There has been a fairly marked improvement in survival over the last few decades [25] over the relatively high mortality reported early on.

Clinical differences between TCA and S-HSCR

The first area of difference is that, although TCA, like S-HSCR, presents with a functional intestinal obstruction, the mode of presentation appears to differ between the two. The initial presentation is often as a functional obstruction at or shortly after birth, but a later presentation is not uncommon in TCA. Presentation within the first few weeks of life [24] is in keeping with the severe clinical picture but TCA, not infrequently, may have a milder presentation much later than would be expected when the length of the aganglionic segment is considered. A number of late presenting TCA cases have been reported [26–29] suggesting that the underlying pathophysiology may differ from the more common form of short-segment Hirschsprung's disease (HSCR). There are even a number of reports of TCA presenting as late as adolescence and early adulthood [27–29].

Our own findings are in keeping with this observation and a later than expected presentation was observed in 9 (27 %) children with TCA who presented outside the Neonatal period (8 presenting >6 months (14 %) and 2 (2 %) >12 months!) [7].

Secondly, TCA may be difficult to diagnose, posing certain difficult management problems prior to and after definitive surgery. In our series, this applied to 50 % of cases with two patients requiring a re-siting of their stoma due to an incorrect initial assessment of aganglionic length [8]. The nature of the difficulties encountered in diagnosis will be dealt with in the sections below, but one of the factors potentially influencing diagnosis is the length of small bowel involvement in any given case.

Radiological features

The sensitivity and specificity of a contrast enema in the overall diagnosis of HSCR is reported as being 76 and 97 %, respectively [30]. By way of contrast, it has long been appreciated that the diagnosis of TCA may differ from this and an accurate determination of a transition zone has been reported in as few as 25 % patients with TCA [31]. It is often a particularly difficult radiological diagnosis, particularly in newborn infants because of lack of consistency in the X-ray findings (Fig. 1a, b) [12]. In this regard, a false transition zone has been reported in the sigmoid in a number of cases. Partly, this is because the colon may appear normal on contrast studies and the radiological findings may be also influenced by the length of small bowel involvement of a particular case. In this regard, the presence of distended small bowel loops on the plain abdominal film may be suggestive.

Despite these difficulties, distinct patterns of radiological features are being identified which may indicate the likelihood of TCA being present. A more recent study [32] identified 3 distinct types of radiological pictures in TCA on contrast enema [viz: microcolon, the question mark-shaped colon and the lack of features in an otherwise normal colon]. Early studies suggested that the retention of barium >24 h was strongly suggestive of HSCR [12, 33]. However, the use of Barium has largely given way to water soluble contrast in modern practice, making it a less practical indicator.

An additional factor is that the clinical and radiological findings of TCA and allied disorders (e.g. Hypoganglionosis) are similar in neonates and may be difficult to separate from TCA [34].

It therefore stands to reason that the diagnosis of TCA must be entertained if clinical symptoms of intestinal obstruction persist in the absence of any other known causes despite a radiologically normal-looking colon.

Histological differences in TCA

There are a number of issues related to differences in the histological features in TCA which may lead to difficulties in diagnosis.

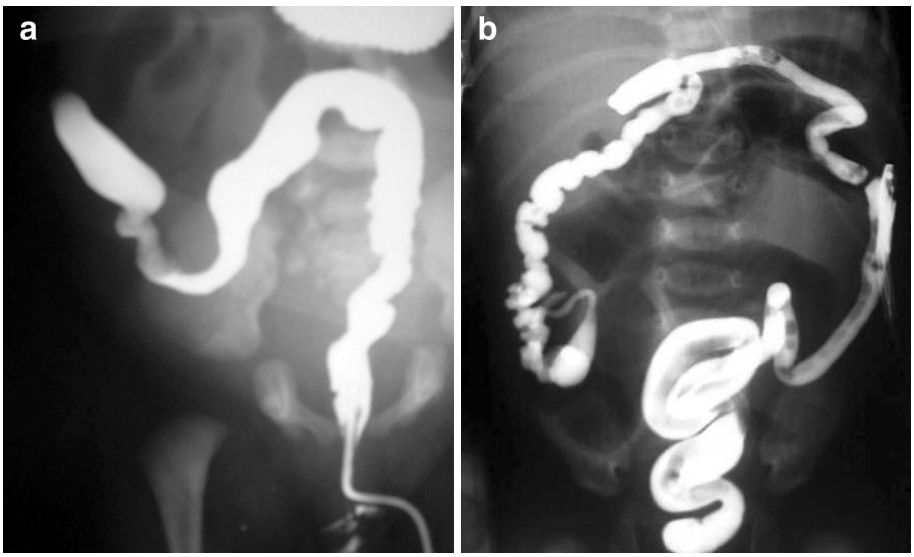

Fig. 1 **a** A contrast enema showing normal caliber colon in a neonate with TCA. **b** A contrast enema showing a microcolon in a patient with TCA

Firstly, frozen section pathologic evaluation may be misleading. In our series the transition zone was mistaken on frozen section due to the presence of abnormal cells leading to re-operation in two of four cases where difficulty in frozen section evaluation was experienced.

Secondly, the expected histological picture for a diagnosis of HSCR may not be as obvious due to an abnormal bowel enervation and ganglion cell populations in TCA [35].

Thirdly, the presence of thickened nerve trunks has been reported to differ from that of short-segment aganglionosis and may be completely absent in TCA bowel [36–38].

In addition, other neural elements may also be deficient in the intestinal wall in TCA. There is some evidence of a moderate hypoplasia of extramural sympathetic innervation as well as the cells of Cajal in the intestinal wall of TCA patients [39]. Additional studies have shown that although the reduction of interstitial cells of Cajal occurs in both short- and long-segment HSCR on Kit staining [39], there is an almost a total lack of all 3 types (submucosal, longitudinal muscle and myenteric plexus) of ICC in the TCA samples investigated suggesting a very severe effect on intestinal motility [35]. Solari and Puri [35] also noted that in addition to the aganglionosis, a markedly reduced Peripherin immunoreactivity and a markedly reduced number of NADPH-positive nerve trunks were present in these patients.

Although the presence of a long hypoganglionic segment and increased immaturity of cells reported in some animal models [40], an extended hypoganglionic segment has not been confirmed in humans (although suspected). If present, it may have an influence on post-surgical functional outcome. All of these may confound the histological diagnosis of TCA.

Pathogenesis and etiology of TCA

Although there has been a significant increase in knowledge and understanding of the pathogenesis of HSCR and TCA over the last 2 decades [25], there are a number of differences from other forms of HSCR which require explanation if its ubiquitous clinical features are to be understood. HSCR itself is characterized as a sex-linked heterogonous disorder with variable severity and incomplete penetrance giving rise to a variable pattern of inheritance [41]. Alterations of the major susceptibility genes (RET and EDNRB) have thus far only been demonstrated in 30–50 % of patients with HSCR generally which is higher than the expected in the normal population [2, 42–44]. In addition, these genetic variations account for more than 50 % of the observed abnormalities associated with HSCR. A wider analysis of these genes to include the early introns and promoter regions increases this figure considerably.

It is, however, not yet clear whether TCA merely represents a long form of HSCR or a different expression of the disease. For one thing, the clinical and histological findings suggest a distinct difference in enervation which is not easily explained on the basis of an increased gene penetrance alone. There is also some evidence suggesting that in place of being purely congenital, TCA may represent certain different pathophysiologic mechanisms, some of which may continue to be active after birth due to continued plasticity of the ENS.

Animal models of TCA

Major contributions have been made to increase our understanding of the ENS by the study of the

developmental processes that contribute to ENS development in animal models.

Examples of animal models of HSCR include both murine [e.g. the lethal spotting mouse (point mutation EDN3), Piebald lethal mice (sl: absent EDNRB)] and rodent [spotting lethal rat (301 bp EDNRB del) and the Dominant Megacolon (Dom; point mutation SOX10) models.

Early animal model appears to have limited aganglionic lengths [45, 46]. However, subsequent models such as the autosomal recessive spotting lethal rat [endothelin-B receptor (EDNRB) gene deletion that prevents functional EDNRB receptor expression] demonstrate two lengths of aganglionosis (i.e. mid-colon and TCA). Only the sl rodent model (EDNRB -/-) has produced TCA fairly consistently. Nagahama et al. [47, 48] showed a paucity of myenteric and submucosal nerve fibers in the affected intestine of these animals whilst bundles of irregular nerve fibers without ganglion cells were present in the circular muscle layer of the mid to distal colon.

It is generally accepted that genetic mutations in these animal models result in developmental defects in neural crest cell migration, differentiation or survival. On the other hand, transgenic expression has been shown to be able to prevent aganglionosis in these animals [49].

Numerous knockout (KO) models have been developed which include the RET ligands GDNF, GFRα1-2, Neurturin, those affecting the endothelin pathway (e.g. EDN3, ECE-1 and EDNRB) and the hedgehog pathways (IHH and SHH). Those KO models affecting the RET ligands GDNF and GFRα tended to produce total intestinal aganglionosis along with those related to SOX10, PHOX 2B and PAX3 making them likely molecular targets.

The endothelin system has clearly been shown to be one of the important genetic factors in the pathogenesis of aganglionosis (e.g. the sl rodent EDNRB -/- phenotype) but its significance in TCA in humans is as yet not completely clear. It would appear, however, that on the basis that a balanced, coordinated interaction between the Sox10 and EDNRB genes is necessary for normal ENS development, that they both may be involved in TCA pathogenesis.

The transcription factor Sox10 has been shown to be required for proper development of a number of neural crest-derived cell types (including melanocytes and both autonomic and enteric neurons). All subtypes of peripheral glia are also absent in mice homozygous for Sox10 mutations. Kapur [50] concluded from studies on the Sox10 (Dom)/Sox10 (Dom) genetic animal model, that excessive cell death occurs in neural crest cells early in the development in these animals due to an early increase in neural crest cell apoptosis rather than defects in the enteric microenvironment. In this model, whereas mutant crest cells did not colonize the Sox10 (Dom)/Sox10 (Dom) gut, explanted segments of Sox10 (Dom)/Sox10 (Dom) embryonic intestine were colonized by wild-type neural crest cells. In addition, apoptosis was increased in early neuroblast cell development in Sox10 (Dom)/Sox10 (Dom) embryos, prior to them colonizing the intestine. Also, double SOX10 mutants demonstrate even more severe ENS defects without signs of apoptosis, cell proliferation or overall neuronal or glial differentiation, which suggests that SOX10 may potentially play a vital role in the apoptosis associated with TCA [51].

Technological advances have allowed the addition of knockout animal models as well as genome-wide searches for profiling gene expression in both wild-type and mutant animal models of the ENS to identify important molecules which play a significant role in enteric neurogenesis [52]. The second advance of using multipotential ENS progenitors as novel therapeutic strategies is currently under scrutiny [53].

An extended transition zone in TCA?

Many of the HSCR animal models demonstrate an extended transition zone or region of hypoganglionosis. TCA has been reported in the Dominant megacolon mouse (Dom) along with a long hypoganglionic transition zone [54]. These cells may also remain immature beyond an age when they should be mature [40]. This is also reported in the murine-16 animal model of DS-HSCR [55] whether this may occur in humans is still not proven although suspected.

Genetic profile of TCA

HSCR is widely regarded as a genetic, sex modified, multifactorial condition with a variable severity and incomplete penetrance of a number of genes. Essentially, HSCR appears to result at a molecular level from disruption of normal signaling during development. The cues controlling the migration of the neural crest cells go awry resulting in aganglionosis of the distal bowel. The disorder is complex, as is shown by the number of genes implicated in its pathogenesis (at least 12). This is hardly surprising as the signals governing cell migration and development in the embryo are extraordinarily complicated and signaling molecules are notorious for crosstalk and redundancy, as well as having coordinate and dependent regulation of expression on occasion.

The genetic profile of TCA is as yet not completely clear although current research would suggest possible different signaling pathways in its pathogenesis. It would appear that the genetic influence varies in terms of the length of the affected segment. TCA is much more common in familial series ($P < 0.001$) [18] which suggests a probable genetic

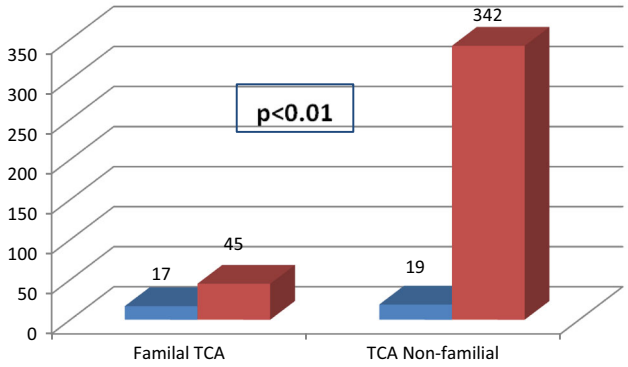

Fig. 2 A comparison between the prevalence of TCA in familial cases versus a non-familial group demonstrating the significantly higher prevalence in familial cases

link (Fig. 2). This is in keeping with the findings of Badner et al. [41] who reported a high degree of heritability and gene penetrance in TCA. Certain families also show increasing length with successive siblings [18] which suggests increased gene penetrance with successive generations. Other modifying genes may influence this phenomenon.

In terms of Mendelian inheritance, autosomal dominant, recessive and polygenic patterns have all been reported in HSCR, particularly where longer segments are observed [18]. Generally speaking, long-segment Hirschsprung's disease and TCA, appears to have an autosomal dominant inheritance pattern with incomplete penetrance [56] (mostly RET), whereas short-segment Hirschsprung's disease appears to be transmitted in an autosomal recessive manner. The heterogeneity of RET proto-oncogene has also been well established in autosomal dominant forms of HSCR [57]. This difference introduces the possibility of different underlying genetic and molecular mechanisms in the pathogenesis of the different phenotypic expressions. In other words, there may be a different genetic profile in TCA than that of S-HSCR in terms of gene penetrance or the multiplicative effects of a number of involved genes [58].

The RET and EDNRB signaling cascades remain the two major susceptibility pathways for HSCR and TCA. RET has been shown to be the main susceptibility gene in TCA, having been associated with the first classic description of RET in association with HSCR [59]. Despite the fact that RET has been identified as the main TCA susceptibility gene [43], animal models of EDNRB [60, 61], PHOX2B mutations [62] and possibly SOX10 [51] have also been implicated. In addition, the position of the genetic variations on the RET gene may influence other signaling pathways thus creating the resultant phenotype. This should lead to further research as to other potential gene–gene interaction to explain these phenomena. In terms of the multiplicity of genes potentially involved in HSCR pathogenesis, there is evidence to suggest that RET is a possible final common pathway to their influence on the developing ENS.

The main question is whether the genetic profile of these patients offers clues as to the reasons for the different pathogenesis in these patients. In our own TCA study [8], RET variations were detected in 82 % with 50 % of these having multiple genetic RET variations. Multiple RET variations were, however, observed in more or less the same proportion in both S-HSCR and extended colon involvement. Despite this, the genetic variation appeared to be more extensive in five suggesting that increased gene penetrance may account for many TCA phenotypes. There is also, however, increasing evidence that disturbances of downstream RET-related signaling pathways may influence the phenotypic expression. It is in this context that further signaling modification by aberrant downstream pathways remains a strong possibility. The pattern of RET gene variation appeared to be less consistent in TCA with a less frequent association with the important exon two variations (A45 polymorphism) than in those with L-HSCR and S-HSCR but was an isolated genetic variation in two TCA patients. The clustering of genetic variations to the intracellular portion of the RET gene (particularly exons 17–21) suggests that the position of the genetic variations may be as important as their extent. This observation is supported by Inoue et al. [63] who also identified an increase in RET mutations in the tyrosine kinase domain in 5 (63 %) out of the eight TCA patients with RET mutations. This suggests the possible involvement of other signaling pathways that bind to receptors on those sites [64]. In particular, these binding sites mediate the recruitment of phosphotyrosine-binding domain-containing adaptor proteins which, in turn, appear to promote the relocation of RET receptor complexes to lipid rafts, thereby promoting downstream signaling and RET-mediated cellular functions [65].

Recently it has been shown that diminished RET expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice suggesting once again that apoptotic mechanisms may be important [66]. Modulation of this mechanism may be of considerable interest in the future treatment of HSCR by modulating RET to be neuroprotective and override the apoptotic mechanisms involved in RET insufficiency [67].

The endothelin system is also important but its significance in TCA is as yet unclear. Colonic ENS development appears to be specifically related to EDN3 [68] and a reduced EDN3 mRNA expression has been reported in the aganglionic segment [69]. Our own study [8] showed EDNRB exon four variations in 32 %, but the significance of this finding is as yet unclear but many of the genes identified in HSCR pathogenesis are interlinked.

In addition, a Cysteine radical mutation (C620R) in a patient with TCA was related to MEN2 in the family in 2 further patients in our study cohort [8]. The co-segregation of the multiple Endocrine neoplasia syndrome (MEN) and HSCR(MEN–HSCR) is highest in patients with long-segment HSCR and a *C620* mutation and has been reported in as many as 54 % of patients [70] and has been a constant association in all of our familial MEN–HSCR cases [18, 71].

One possible explanation for TCA is, therefore, that immature ganglion cells may still possibly be influenced and processes such as apoptosis or alternatively, death of ENS cells [50], may still continue after birth. It is thus possible that some degree of postnatal ENS plasticity may contribute to and possibly explain the histological differences observed in this and other studies [35]. This also provides a potential reason for the degenerating cells and "ghost" ganglion cells observed on histology in two of our cases [8]. Further support for this hypothesis also comes from experiments showing early death of neural crest cells in the Sox10 (Dom)/Sox10 (Dom) experimental murine animal [50] and suggests a genetic cause.

In addition to Sox10, the related *NRG1* gene at 8p 12 which encodes neuregulin 1 is also a candidate being involved in regulating enteric neural precursor development. Sox10 is also a pre-requisite for NRG1-dependent survival of the multipotent neuroblasts colonizing the ENS especially during gliogenesis [72]. Further genome-wide associations have also identified the NRG locus as an additional significant susceptibility locus in HSCR in humans [73].

Associated conditions

A number of developmental conditions have been associated with TCA [40], along with several known syndromes inherited in an autosomal dominant manner. These include chromosomal [59] and congenital hypoventilation syndrome (with a PHOX2B gene mutation [62]) as well as ileal atresia [74, 75] and tumors of neural origin [76]. Although the pattern of conditions associated with HSCR has already been of great value in revealing many of the genetic nature and associations of the disease [77, 78], there appears to be no consistent association with specific anomalies and TCA. In fact, there appears to be a decreased TCA incidence (6 %) associated with Trisomy 21 [24, 25, 79] as opposed to the known higher occurrence of HSCR.

Outcome following surgery

Many different surgical techniques have been utilized for TCA [14, 80, 81] with outcomes mostly related to the type of surgical technique performed [25]. Those used include the Soave, Swenson, and Duhamel and Martin [14] techniques or the Kimura colonic patch [81, 82]. In a 30-year survey of TCSA in Japan, Ieiri et al. [15] noted that Duhamel procedure and colonic patch methods have increased over time to replace the Martin-extended Duhamel and other procedures because of non-optimal results or specific procedure-related problems. Many surgeons now accept the standard modified Duhamel procedure as the best option in TCA in terms of long-term function. [25].

In a fairly recent study of outcome in 58 patients [83] it was found that surgical management of TCA was largely successful. Although mortality in TCA has decreased dramatically in recent years the morbidity still remains high [21]. In the large Japanese series [15], although the mortality dropped from 40.9 to 15.8 %, a high mortality still encountered. These are particularly severe in those with extensive small bowel involvement for fairly obvious reasons. Nevertheless, TCA patients continue to have long-term issues with bowel control and night diarrhea. Although some of these may improve with time [15, 25], the long-term follow-up of 42 (2–31 years) TCA patients [83] showed that although 22 (52 %) had good bowel control, continence remained a problem in the remaining 20 (47 %). These patients averaged 5.2 bowel movements per day which decreased to a mean of 3.4 per day at the age of 15 years. Ikawa et al. [84] identified severe iron deficiency and growth retardation in these patients.

Hirschsprungs-associated enterocolitis (HAEC) appears to remain a problem in patients with TCA, being identified postoperatively in 55.4 % [83] of one series in keeping with other long-term follow-up studies [6, 10, 12, 26, 80, 85]. Ieiri et al. [15] demonstrated a significant decrease in HAEC in recent years. This requires careful evaluation as it may mean that the frequent stools encountered in many were attributed to the short bowel rather than HAEC as in the past. Additional mechanical problems have been experienced in older patients due mostly to the "kinking" of the small bowel in the pelvis which may require further surgical management.

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