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Total colonic aganglionosis in Hirschsprung disease

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Total colonic aganglionosis (TCA) is a relatively uncommon form of Hirschsprung disease (HSCR), occurring in approximately 2%-13% of cases. It can probably be classified as TCA (defined as aganglionosis extending from the anus to at least the ileocecal valve, but not >50 cm proximal to the ileocecal valve) and total colonic and small bowel aganglionosis, which may involve a very long segment of aganglionosis. It is not yet clear whether TCA merely represents a long form of HSCR or a different expression of the disease. There are many differences between TCA and other forms of HSCR, which require explanation if its ubiquitous clinical features are to be understood. Clinically, TCA appears to represent a different spectrum of disease in terms of presentation and difficulties that may be experienced in diagnosis, suggesting a different pathophysiology from the more common forms of HSCR. There is also some evidence suggesting that instead of being purely congenital, it may represent certain different pathophysiologic mechanisms. This study, in addition to reviewing current understanding and differences between TCA and the more frequently encountered rectosigmoid (or short-segment) expression, correlates them with what is currently known about the genetic and molecular biological background. Moreover, it reviews current outcomes to find consensus on management.

Hirschsprung disease (HSCR) presents as a low intestinal obstruction due to the congenital absence of the intramural plexuses of ganglion cells (aganglionosis) in the distal bowel. It varies in length and is classified into the clinical groups of ultra-short-segment, short segment (S-HSCR), and long segment (L-HSCR).[1,2] Long-segment disease can be further divided into long-segment colonic aganglionosis, total colonic aganglionosis (TCA), and total colonic and small bowel aganglionosis (TCSA). The latter may involve a very-long-segment HSCR (Zuelzer syndrome), and there are reports of total bowel aganglionosis.[3-6]

TCA may be regarded as separate from the extended intestinal form (TCSA) as well as the very rare form of aganglionosis that stretches from duodenum to anus.[5,6] Because of the presentation and special problems associated with very long aganglionic segments, it would appear logical to define TCA as aganglionosis extending from the anus to at least the ileocecal valve, but not >50 cm proximal to it.[7,8] Longer aganglionic segments pose special management problems and could be regarded as a separate group. It is not yet completely clear whether separation of these 2 entities is justified in terms of pathogenesis and biology, requiring further research.

TCA is an uncommon HSCR phenotype, occurring in approximately 2%-13% of cases.[9,10] In the Japanese population, the incidence of TCA averaged 1 in 58,496 individuals, with a male:female ratio of 1.5:1 over a 30-year period.[11] Other studies have shown a male:female ratio in TCA approaching an almost equal sex occurrence.[12]

TCA has long been recognized as presenting particular problems in diagnosis[13,14] and management.[15-19] More recently, the concept is emerging that HSCR represents a spectrum of conditions producing functional intestinal ob-

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struction, which have aganglionosis of the intermyenteric plexuses as a common feature. The benefit of this wider concept rests on the better understanding of the pathogenesis and genetic connections of the disease, including TCA.

Pathogenesis of aganglionosis

It is generally accepted that intestinal aganglionosis occurs as a result of the aberrant colonization of the enteric nervous system (ENS) neuroblasts during development.[20,21] Although HSCR is largely regarded as a genetic-derived condition, research has identified at least 5 levels where altered molecular signaling potentially contributes to the pathogenesis of HSCR. These include a decreased pool of available neuroblasts for migration into the ENS in syndromic cases (eg, Down syndrome),[22,23] early gene-related influences on the migrating neuroblasts,[20,21] abnormally developed neuroblasts migrating into ENS (eg, Down syndrome),[24] germline and somatic mutations of genes,[25-28] and alterations in the local tissue environment.[29] It would appear that all these contribute to the disruption of normal signaling during enteric neuroblast development.

Familial recurrence

It is well known that affected families carry an approximately 200 times higher risk of HSCR recurrence than normal population.[10,30] This familial recurrence tends to demonstrate a higher prevalence of longer aganglionic segments and TCA, presumably due to increased gene penetrance. The study by Badner et al[31] reported a high degree of heritability and gene penetrance in TCA, and it would appear that the longer the aganglionic segment, the higher the risk of familial recurrence. We have also shown a significantly ($P < 0.001$) higher TCA prevalence in familial HSCR (Figure 1).[10,32,33] The corollary of this is also true, and patients with long-segment aganglionosis (L-HSCR) have a demonstrable significantly higher family risk[10,32,33] of the order of 15%-21%.[34] The relative risk increases to almost 50% in patients with ultra-long-segment aganglionosis (TCSA).[35] In addition, a progression of severity through subsequent generations has been reported, indicating increased gene penetrance.[10]

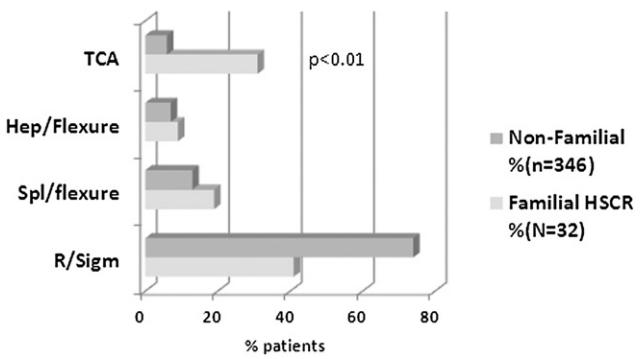

Figure 1 A comparison of aganglionic segment length: familial (n = 32) versus nonfamilial (n = 346) Hirschsprung disease.

Clinical differences between TCA and S-HSCR

TCA is generally regarded as a special problem area in the HSCR spectrum of disease because of suboptimal long-term results and frequent complications.[19] Although TCA shares the common feature of aganglionosis with other forms of HSCR, it differs in several respects, which supports the concept that it is part of a collection of conditions related to the abnormal development of the ENS.

First, it is much more common in female individuals, and the 4:1 male predominance of S-HSCR decreases to 1.1:1 or even 0.8:1 for TCA.[16,33,36]

Second, TCA appears to represent a different spectrum of disease in terms of clinical presentation; although TCA, like S-HSCR, presents with a functional intestinal obstruction, the mode of presentation often differs. Although most present with an acute clinical picture in the first few weeks of life,[36] as the extensive disease would suggest, it not infrequently presents in a milder, more subtle form possibly much later than expected.[37-41] In addition, there are many reports of TCA presenting as late as adolescence and early adulthood.[37-39] Our own findings are in keeping with this, and a later-than-expected presentation was observed in 9 (27%) TCA neonates after the neonatal period (8 presenting after >6 months (14%) and 2 (2%) after 12 months).[42]

As a result, difficulties may be experienced in the diagnosis of TCA,[8,19] posing certain difficult management problems before and after definitive surgery. As a result, some have even gone as far as to suggest that it be regarded as a separate condition from the usual S-HSCR.[8] In our series, difficulties in diagnosis were encountered in 50% of cases. In particular, 2 patients required resiting of their stoma owing to an incorrect assessment of the aganglionic bowel,[41] in common with other series.[19]

Although there has been a marked improvement in survival of TCA patients over the past few decades,[43] it is becoming clear that many of the results of TCA are suboptimal[19] and present many challenges. One of the factors potentially influencing the difficulties encountered in diagnosis is the condition and length of small bowel involvement in any given case. This suggests that the underlying pathophysiology may differ from the more common form of S-HSCR and will be further explored in the sections later in the text.

Radiological difficulties

The diagnosis of TCA is particularly difficult to make in newborn infants because of lack of consistency in the ra-

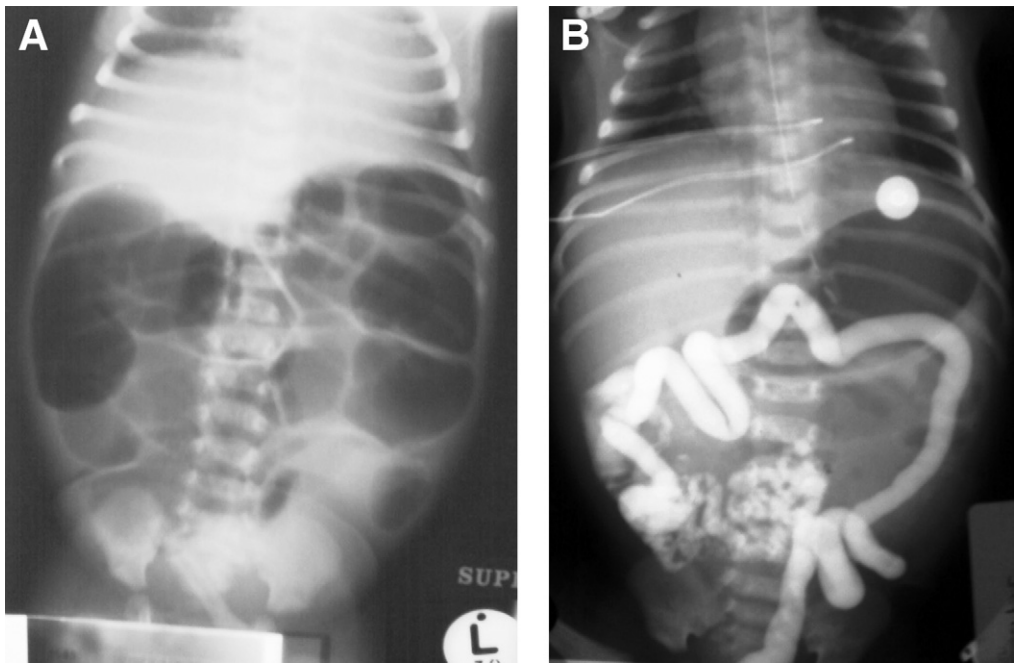

Figure 2 (A) Abdominal radiograph (supine) of a patient with TCA, demonstrating obstruction, and (B) contrast enema of same patient demonstrating unused small colon.

diological findings[16] (Figure 2), which are influenced by the length of small bowel involvement. Sensitivity and specificity of a contrast enema in the diagnosis of HSCR are reported as being 76% and 97%, respectively,[44] but may be extremely difficult in TCA, with a transition zone only being accurately determined in as few as 25% of TCA patients.[45]

As a result, false transition zones may be reported as being in the sigmoid.[16] Early studies suggested that the retention of barium for >24 hours was strongly suggestive.[16,46] In a more recent study,[47] 3 types of radiological picture in those with TCA were observed: microcolon, the question mark–shaped colon, and the lack of features in an otherwise normal colon; the classic question mark shape was observed in only 18% of children. The colon may appear normal on contrast studies, and the diagnosis of TCA must be entertained if clinical symptoms of obstruction persist in the absence of any other known causes.

Histologic differences in TCA

The ENS changes in TCA are quite extensive, and it has recently been shown that expression levels of the nerve marker immunostains are significantly ($P < 0.01$) decreased in patients with TCA when compared with those with less severe HSCR.[48] This observation is probably explained by the differences in enteric nerve innervation and the relative paucity/absence of thick nerve trunks in TCA bowel, and has practical significance because it may lead to diagnostic difficulties. This is illustrated in our own series where the transition zone was mistaken on frozen section owing to the presence of abnormal cells, which led to repeat procedures in 2 of 4 cases where diagnostic difficulty was experienced.

In addition to changes in bowel enervation and abnormal ganglion cells, abnormalities in the interstitial cells of Cajal (ICC) have also been reported.[49,50] The reduction in the number of ICC observed in both S- and L-HSCR appears to be particularly prominent in TCA,[50] with almost a total lack of all 3 ICC types (submucosal, longitudinal muscle, and myenteric plexus). This suggests a very severe effect on intestinal motility in these patients.[50]

In addition to the aganglionosis, a moderate hypoplasia of extramural sympathetic innervation has been reported in those with TCA along with reduced peripherin immunoreactivity and a markedly reduced number of NADPH-positive nerve trunks.[49]

In addition to raising the question of difficulties that such changes may create in diagnosing TCA, the wide spectrum of histological changes have a bearing on the normal functioning of the ganglionated bowel. It may also affect results due to possible ongoing motility disturbances due to an extended transition zone in TCA. Possible reasons for this different spectrum of histological features, raises the question of other cellular mechanisms (e.g. apoptosis) being involved in its pathophysiology.

An extended transition zone in TCA?

Many of the HSCR animal models (particularly those with TCA) demonstrate an extended transition zone or region of hypoganglionosis.[51] TCA along with a long hypoganglionic transition zone has been reported in the Dominant megaco-

lon mouse (Dom)[52] as well as the murine-16 animal model of down syndrome associated Hirschsprungs disease.[53]

The long hypoganglionic segment with increased immaturity of cells reported in some animal models,[51] has not been confirmed in humans (although suspected). Nevertheless, immature ganglion cells in the transitional segment have been reported in TCA.[48] This may have an influence on post-surgical outcome and patients with TCA could experience ongoing motility problems following otherwise successful surgery which may well be related to a retained abnormal proximal small bowel. This is also a feature of certain types of syndromic HSCR (eg, Mowat–Wilson syndrome), where motility problems are pronounced.

Associated congenital anomalies and TCA

A number of developmental conditions have been associated with TCA,[51] along with several known syndromes inherited in an autosomal-dominant manner. Although the pattern of conditions associated with HSCR has already been of great value in revealing many of the genetic natures and associations of the disease,[54,55] there appears to be no consistent association with specific anomalies and TCA. Associations with chromosomal abnormalities (including Down syndrome)[56,57] and other syndromes such as the congenital hypoventilation syndrome (with a paired-like homeobox 2b [*PHOX2*] gene mutation[57]) as well as associations with other congenital anomalies (eg, ileal atresia[58,59]) and tumors of neural origin[60] indicate a probable genetic mechanism. However, in contrast to the known higher occurrence of HSCR in those with Down syndrome, TCA appears to be uncommon in those with trisomy 21 ($\pm$6%).[36,43,61]

Pathogenesis and possible etiology of TCA

There has been a significant increase in knowledge and understanding of the pathogenesis of HSCR and TCA over the past 2 decades,[43] but it is not yet clear whether TCA merely represents a long form of HSCR or a different expression of the disease. One of the reasons is that the variable clinical and histologic findings suggest distinct differences in enervation, which cannot be easily explained on the basis of an increased gene penetrance alone. There is some evidence suggesting that instead of occurring purely as the result of impaired colonization of the ENS, it may represent a number of pathophysiologic mechanisms (eg, maturation, differentiation, apoptosis, etc), some of which may continue to be active after birth owing to continued plasticity of the ENS.

Genetic profile of TCA

Although it is generally accepted that HSCR is multifactorial in nature,[62] genetic factors are recognized as playing a major role in its pathogenesis. Many, if not most, of the pathogenetic factors are related to a variable penetrance of a number of genetic variations (at least 11 genes), which determine the final phenotypic expression.[30,63]

TCA associations with the main gene signaling pathways

The majority of genes implicated in HSCR pathogenesis are related to 1 of the 2 main gene susceptibility pathways identified (the REarranged during Transfection [RET; RET, glial cell line derived neurotrophic factor (*GDNF*), glial cell line derived neurotrophic factor family receptor alpha (GFRα), neurturin (*NTN*)] signaling cascade and the endothelin B receptor–related pathways [endothelin receptor type B (*EDNRB*), endothelin 3 *(EDN3)*, endothelin converting enzyme 1 (*ECE-1*), *PHOX2*, and SRY-box containing gene (*SOX10*)]). Other identified genes are mostly related to specific syndromes, and their pathogenetic connection to HSCR is not as yet fully understood. It is therefore important that the extended form of the condition in 2%-13% of patients[7-9] be explored, to assist in genetic counseling, particularly in potential familial recurrences.

Potential gene effects in the pathogenesis of HSCR within these pathways include loss of function, gain of function, apoptosis, aberrant splicing, and decreased gene expression.[28]

RET gene signaling and TCA

The RET gene signaling system is generally acknowledged as being the most important in TCA pathogenesis, with RET gene variations being present in >70% of cases. A number of studies[28,64,65] have shown that these gene variations may be widespread, as the genetic variations are scattered throughout the gene. Better understanding of these genetic influences shows that loss of function, gain of function, apoptosis, aberrant splicing, and decreased gene expression may all be partly responsible for the final phenotypic expression.[28]

When looking at the genetic profile of TCA, it would appear important to recognize differences between the syndromic and nonsyndromic expressions of the condition. Nevertheless, attempts to identify specific genetic reasons to explain the extended aganglionosis in TCA have until recently been largely unsuccessful. It remains possible that the gene penetrance of HSCR susceptibility genes could be influenced by a number of other modifying genetic, environmental, and possibly local microenvironmental factors.

In our own series of TCA,[41] multiple RET variations were observed in both short- (S-HSCR) and long-segment aganglionosis (L-HSCR). However, these did not appear to be specific features of the extended form, except a clustering of genetic variations in the intracellular portion of the RET gene (particularly exons 17-21) in 8 (33%) of 24 TCA

patients.[8] Subsequent investigation of the RET promoter identified multiple variations in TCA patients, suggesting a further area of susceptibility. In 50% of these patients, these promoter variations were associated with multiple other genetic RET variations. A further area currently being explored is the somatic RET intronic mutations of (*RET* rs2506004 [SNP1] and rs2435357 [SNP2]), which were found to be homozygous in the tissue of a down syndrome associated total colonic aganglionosis patient but heterozygous in ganglionated and transitional bowel segments.[66]

In addition to the multiple variations within the promoter region, the increase in RET mutations in the tyrosine kinase domain (especially exon 17), previously also noted by Inoue et al[67] in 5 of 8 TCA patients (63%), suggests that disturbances in this area may be of significance in L-HSCR. This region is known to contain important binding sites that mediate the recruitment of downstream RET-related signaling pathways that bind to receptors on those sites and which may potentially influence the phenotypic expression.[68] By way of example, disturbance of the phosphotyrosine-binding domain–containing adaptor proteins, which bind in this vicinity, may be involved, as they appear to promote the relocation of Ret receptor complexes to lipid rafts and promote downstream signaling and Ret-mediated cellular functions.[69] Although the genetic changes in this region may be small, there is evidence from other experiments that minor genetic variations in this region may redirect adaptor protein pathways, which may lead to a decrease in cell survival due to signaling modification by aberrant downstream pathways and encourage apoptosis.[69]

The cosegregation of the multiple endocrine neoplasia syndrome (MEN) and HSCR (MEN-HSCR) is highest in patients with L-HSCR. The RET *C620* mutation has been reported in as many as 54% of patients.[70] We have also reported TCA in 2 patients, which was related to the HSCR–medullary thyroid carcinoma association and MEN type 2.[71]

The growing awareness of the different levels of development where altered molecular signaling potentially contributes to the disruption of normal signaling during enteric neuroblast development gives rise to a much wider concept of a multigenic HSCR pathogenesis.

Numerous knockout (KO) models have been developed, including the RET ligands *GDNF*, *GFR*α1-2, neurturin, and those affecting the endothelin pathway (eg, *EDN3*, *ECE-1*, and *EDNRB*) and the hedgehog pathways (Indian hedgehog [*IHH*] and Sonic hedgehog [*SHH*]).

The endothelin system in TCA

The role of the endothelin system in TCA is as yet unclear, but current evidence points toward a significant modifying role in its pathogenesis. First, only the sl rodent model (*EDNRB* −/−) has produced TCA consistently in animal models.[72,73] Second, colonic ENS development beyond the ileocecal valve appears to be specifically related to *EDN3*,[74] and a reduced *EDN3* mRNA expression has been reported in aganglionic segments.[75]

Although significant in animal models, the significance of *EDNRB* gene variations in human TCA is unclear. In our own series, *EDNRB* exon 4 variations was present in 9 of the 24 (37.5%) TCA patients, which is higher than the generally accepted 5% in those with HSCR. The significance of this finding is uncertain, as many of the genes identified in HSCR pathogenesis appear interlinked.[76]

Other potential modifier genes

It is well understood that the effects of common variants in other genes may interact with other alleles or epigenetic factors in HSCR pathogenesis. Technological advances have allowed the addition of KO 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 that play a significant role in enteric neurogenesis.[77]

Research has shown that TCA arises in KO models, affecting the RET ligands *GDNF* and *GFR*α, along with those related to *SOX10, PHOX2B*, and paired box 3 (*PAX3*). More recent research indicates that further genetic modifiers may be present in copy number variants of a wide spectrum of neurodevelopmental genes.[78] This may partially explain the variability reported in the genetic predisposition to HSCR.

Other genes thought to play a role include the ZEB2 gene, which relates to E-cadherin; the TCF4 transmission factor, which works together with *SOX*; the mitochondrial transport–regulating KIAA1279 gene, which relates to the Goldberg Shprintzen syndrome; the neuroglian (*NRG*) antiapoptotic gene, which is part of the mitogen activated kinase-like protein (*MAPK*) cascade; and possibly L1 cell adhesion molecule (*L1CAM*).[78] L1 cell adhesion molecule has recently been shown to act as a modifier gene for members of the endothelin signaling pathway during ENS development.[79]

Therefore, a possible explanation for TCA is that immature ganglion cells may still possibly be influenced and that processes such as apoptosis, or alternatively, death of ENS cells,[80] may still continue after birth. In contrast, the inhibition of cell death results in hyperganglionosis.[81]

It is thus possible that some degree of postnatal ENS plasticity may contribute to and possibly explain the histologic differences observed in this and other studies.[49] This also provides a potential reason for the degenerating cells and "ghost" ganglion cells observed on histology in 2 of our cases.[41] 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[80] and suggests a genetic cause.

Surgical management and outcome of TCA

Many different surgical techniques have been used for TCA,[18,82,83] with outcomes mostly related to the type of

surgical technique performed.[43] Those used include the Soave and Swenson techniques and the "long" Duhamel procedure as modified by Martin.[84,85] In certain parts of the world, the Kimura colonic patch[11,83,86,87] has been a popular method for very long aganglionic segments.

No surgical procedure has clearly been shown to be superior in the management of TCA. Although the Soave and Duhamel procedures have been widely used in the management of TCA, including Duhamel modification to include a long ileal anastomosis (the so-called Lester–Martin procedure),[84,85,88] their popularity is waning because of ongoing problems.

In a 30-year survey of TCSA in Japan,[11] it was noted that use of the Duhamel procedure and colonic patch methods had largely replaced the Martin-extended Duhamel and other procedures in the treatment of TCA. This was because of nonoptimal results or specific procedure-related problems encountered by the surgeon.

Although a comparison of TCA patients managed with the Soave procedure showed fewer operative complications compared with those who underwent the extended Duhamel or Martin procedure,[89] patients managed with the Soave procedure took longer to establish normal defecation.

Kimura et al[86] reported 7 cases where the initial severe postoperative diarrhea was significantly improved by creation of the "patch." Long-term benefit was seen in 4 of these patients during a 5- to 8-year follow-up. This "patch" procedure has probable advantage in cases of extensive aganglionosis involving the colon and distal ileum (5-40 cm).

Many surgeons now accept the standard modified Duhamel procedure as a good option in TCA in terms of long-term function.[43] However, because of technical changes in the modern era and popularization of the transanal approach, many surgeons are now swinging to an ileoanal anastomosis similar to the Swenson procedure, with ileostomy cover,[18] once the level of the ganglionated bowel has been identified. These procedures may be performed in the traditional open manner or (more increasingly) as laparoscopic or laparoscopy-assisted procedures.[90,91] A successful 1-stage laparoscopic Soave procedure has been reported.[92]

Although there is little comparative data on the outcome of laparoscopic surgical approaches, studies on the outcome of similar laparoscopic colectomies have been reported to yield equitable outcomes in pediatric patients as the traditional open method, both in complication type and severity.[91] Patients with a subsequent laparoscopically formed ileal pouch appeared to have a lower incidence of pouchitis. Similar results have been reported for a laparoscopic or laparoscopy-assisted approach to the surgery in those with HSCR.[90]

Although it is possible for patients with TCA to have adequate growth, normal feeding, reasonably good continence, and satisfactory quality of life,[93] a number of problems still remain. A recent long-term follow-up study of 42 TCA patients (2-31 years after surgical correction)[94] found that although surgical management was largely successful, good bowel control was only achieved in 22 (52%), with poor continence control in the remainder. Other studies[11,43] confirmed the long-term issues with bowel control in TCA patients, although some of these may improve with time. Our own studies[12] have shown similar results as well as an incidence of reported "night" diarrhea with the possibility of some leakage. There also appeared to be some related procedure-specific issues with the Martin procedure, possibly due to "kinking" of the extended ileorectal pouch, resulting in intermittent obstructive symptoms. As a result, patients with TCA tend to undergo multiple corrective surgical procedures.[82]

In addition to issues of control, a number of TCA patients may experience poor growth and development associated with severe iron deficiency.[95] In one long-term follow-up study, more than half of the patients were below the second percentile for weight, and one-quarter below the second percentile for height.[82] This raises the interesting question of whether the proximal bowel may or may not be normal in TCA patients.

In the Japanese experience,[11] the TCA mortality was shown to decrease from 40.9% to 15.8% over a 30-year period, although the morbidity still remained high, particularly in those with extensive small bowel involvement.[7,11,19] This emphasizes the need for a clear separation of TCA (small bowel involvement of <50 cm) from ultra-long-segment small bowel aganglionosis (TCSA; small bowel involvement of >50 cm) with regard to evaluating outcome.

TCA and enterocolitis

Enterocolitis associated with HSCR (HAEC) occurs in 16%-58% of HSCR patients and remains a significant cause of morbidity and mortality.[96-98] Although often diagnosed on initial presentation, it may also develop later after surgical correction of HSCR.

HAEC is particularly common in association with TCA, occurring both pre- and postoperatively,[98] contributing significantly to morbidity and mortality.[12,96] HAEC has been particularly associated with L-HSCR, and the occurrence of a postoperative HAEC in one large TCA series during long-term follow-up (2-31 years) was reported in 55.4% (94) of patients, having resulted in 3 patients opting for permanent ileostomy. Ieiri et al[11] demonstrated a significant decrease in HAEC after surgical correction in recent years. However, 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.

Conclusion

The significant differences noted between TCA and rectosigmoid forms of HSCR are probably due to genetic and molecular biological factors. Clinical awareness of these differences is important because of potential pitfalls leading to misdiag-

nosis. Satisfactory postsurgical outcomes can be achieved, but significant challenges still remain.

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