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TOPIC HIGHLIGHT

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Pathogenesis of achalasia cardia

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Abstract

Achalasia cardia is one of the common causes of motor dysphagia. Though the disease was first described more than 300 years ago, exact pathogenesis of this condition still remains enigmatic. Pathophysiologically, achalasia cardia is caused by loss of inhibitory ganglion in the myenteric plexus of the esophagus. In the initial stage, degeneration of inhibitory nerves in the esophagus results in unopposed action of excitatory neurotransmitters such as acetylcholine, resulting in high amplitude non-peristaltic contractions (vigorous achalasia); progressive loss of cholinergic neurons over time results in dilation and low amplitude simultaneous contractions in the esophageal body (classic achalasia). Since the initial description, several studies have attempted to explore initiating agents that may cause the disease, such as viral infection, other environmental factors, autoimmunity, and genetic factors. Though Chagas disease, which mimics achalasia, is caused by an infective agent, available evidence suggests that infection may not be an independent cause of primary achalasia. A genetic basis for achalasia is supported by reports showing occurrence of disease in monozygotic twins, siblings and other first-degree relatives and occurrence in association with other genetic diseases such as Down's syndrome and Parkinson's disease. Polymorphisms in genes encoding for nitric oxide synthase, receptors for vasoactive intestinal peptide, interleukin 23 and the *ALADIN* gene have been reported. However, studies on larger numbers of patients and controls from different ethnic groups are needed before definite conclusions can be obtained. Currently, the disease is believed to be multi-factorial, with autoimmune mechanisms triggered by infection in a genetically predisposed individual leading to degeneration of inhibitory ganglia in the wall of the esophagus.

Key words: Esophagus; Motor dysphagia; Motility disorder; Peristalsis; Esophageal sphincter

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INTRODUCTION

Achalasia is an esophageal motor disorder characterized by aperistalsis of the esophageal body and lack of relaxation of the lower sphincter in response to swallows. It affects both sexes and all age groups[1,2]. Achalasia was first described by Willis[3] in 1674 as "food blockage in

esophagus". He treated these patients successfully with a dilator made of whale bone and sponge[3]. The term "achalasia" was first coined by Hurst[4] in 1927. He had observed such patients since 1914 and suggested that their disorder might be due to absence of normal relaxation of the sphincter, possibly resulting from organic changes in Auerbach's plexus[5]. Achalasia is a Greek word that means "failure of relaxation". Achalasia can be primary (idiopathic) or secondary. In secondary achalasia, the cause for the degeneration of esophageal nerve fibers is known. Pathophysiologically, achalasia is caused by loss of inhibitory ganglion cells in the myenteric plexus. Since the initial description, several studies have attempted to explore initiating agents that may cause the disease such as viral infection, other environmental factors, autoimmunity, and genetic factors. However, the exact pathogenesis of primary achalasia is still not known. In this paper, the literature regarding pathogenesis of primary achalasia is reviewed.

PATHOPHYSIOLOGICAL ISSUES

The pathophysiology of achalasia is outlined in a simplified manner in Figure 1. In healthy esophagus, progressive delay in contractility of the lower esophageal muscles results from the presence of inhibitory neurotransmitters such as nitric oxide and its receptors in the lower esophagus (Figure 1)[6,7]. In the initial stage of the disease, degeneration of inhibitory nerve fibers in the esophagus results in unopposed action of excitatory neurotransmitter such as acetylcholine, which leads to high amplitude non-peristaltic contractions (not progressively delayed or simultaneous)[8]. This stage of achalasia is known as vigorous achalasia (average amplitude of contractions in lower esophagus > 40 mmHg)[9]. Progressive loss of cholinergic neurons results in dilation and low amplitude simultaneous contractions in the esophageal body; this stage of achalasia is called classic achalasia. Studies demonstrating reduction in number of ganglion cells in the esophageal body at autopsy of patients with achalasia and an inverse correlation between number of ganglion cells and duration of disease support their involvement in the disease process[8]. In experimental studies published long ago, a muscles strip obtained from the esophageal body of patients with esophageal achalasia failed to contract on addition of the ganglion stimulant nicotine though it contracted in response to acetylcholine, which is a direct muscle stimulant. A muscle strip from the lower esophageal sphincter (LES) of patients with achalasia contracted in response to acetylcholine though it failed to relax in response to nicotine[10]. These findings demonstrate that in patients with achalasia, there is degeneration of ganglia though the muscles remain contractile in response to acetylcholine. Degeneration of inhibitory control of the LES has also been demonstrated by studies that showed cholecystokinin, which reduces LES pressure in healthy subjects, increases pressure in patients with achalasia[8]; esophageal distension failed to cause relaxation of LES in these patients[11] and gastric distension failed to induce transient LES relaxation[12]. All the above data suggest that achalasia results from degeneration of the esophageal nerve plexus, particularly the inhibitory fibers. However, neural degeneration might not be confined to the esophagus alone as evidenced by demonstration of Wallerian degeneration in the vagus nerve on electron microscopy[13], improvement in some abnormalities with stimulation of vagus nerve in animal models[14,15], presence of Lewy bodies in the brainstem of patients with achalasia[16], and delayed gastric emptying[17] and abnormal gastric secretory response to insulin-induced hypoglycemia in a subset of patients[18]. It is, however, not clear why some people develop neural degeneration causing achalasia. The various environmental, autoimmune and genetic factors incriminated in pathogenesis are reviewed below.

A study in an animal model showed that the nitric oxide inhibitor, recombinant human hemoglobin, caused incomplete LES relaxation and high amplitude simultaneous contractions in the body of the esophagus[19]. Similar results have been reported in humans[20]. Another study in an animal model failed to show an association of intramuscular interstitial cells of Cajal in the nitrenergic pathway and dysfunction of LES found in achalasia[21].

Infection

A number of studies implicating viral agents in the pathogenesis of achalasia showed conflicting results. An initial report by Jones *et al*[22] showed a statistically significant increase in antibody titer against measles virus (MV) in patients with achalasia compared with controls (14 of 21 achalasia patients *vs* 7 of 21 controls, $P < 0.05$). This difference persisted only at a titer of 1/32 or more. However, there was similar frequency of infection in both groups as was evident by similar antibody detection rates at low titer[22].

Robertson *et al*[23] reported an association between Varicella zoster virus (VZV) infection in patients with achalasia by demonstrating viral DNA in esophageal tissue. They demonstrated VZV particles by DNA hybridization techniques in three of nine esophageal myotomy specimens from achalasia patients, but in none from 20 control subjects. DNA probes for cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1) were negative in both achalasia patients and controls[23]. This study provided an attractive hypothesis, but failed to establish a causal association. We have reported one patient who presented with motor dysphagia due to esophageal hypomotility and also developed gastroparesis following infection with VZV[24].

A few studies, however, failed to show evidence of infection as a cause for achalasia[25,26]. Niwamoto *et al*[25] used polymerase chain reaction to detect human herpes virus DNA (HSV-1 and 2, CMV, VZV, Epstein-Barr virus and human herpes virus-6) or MV RNA in the esophageal muscle of 12 patients with achalasia and six with upper esophageal carcinoma. Only HSV-1 and 2 were detected in all samples including patients and controls. Other viruses were not detected. Similarly, another study failed

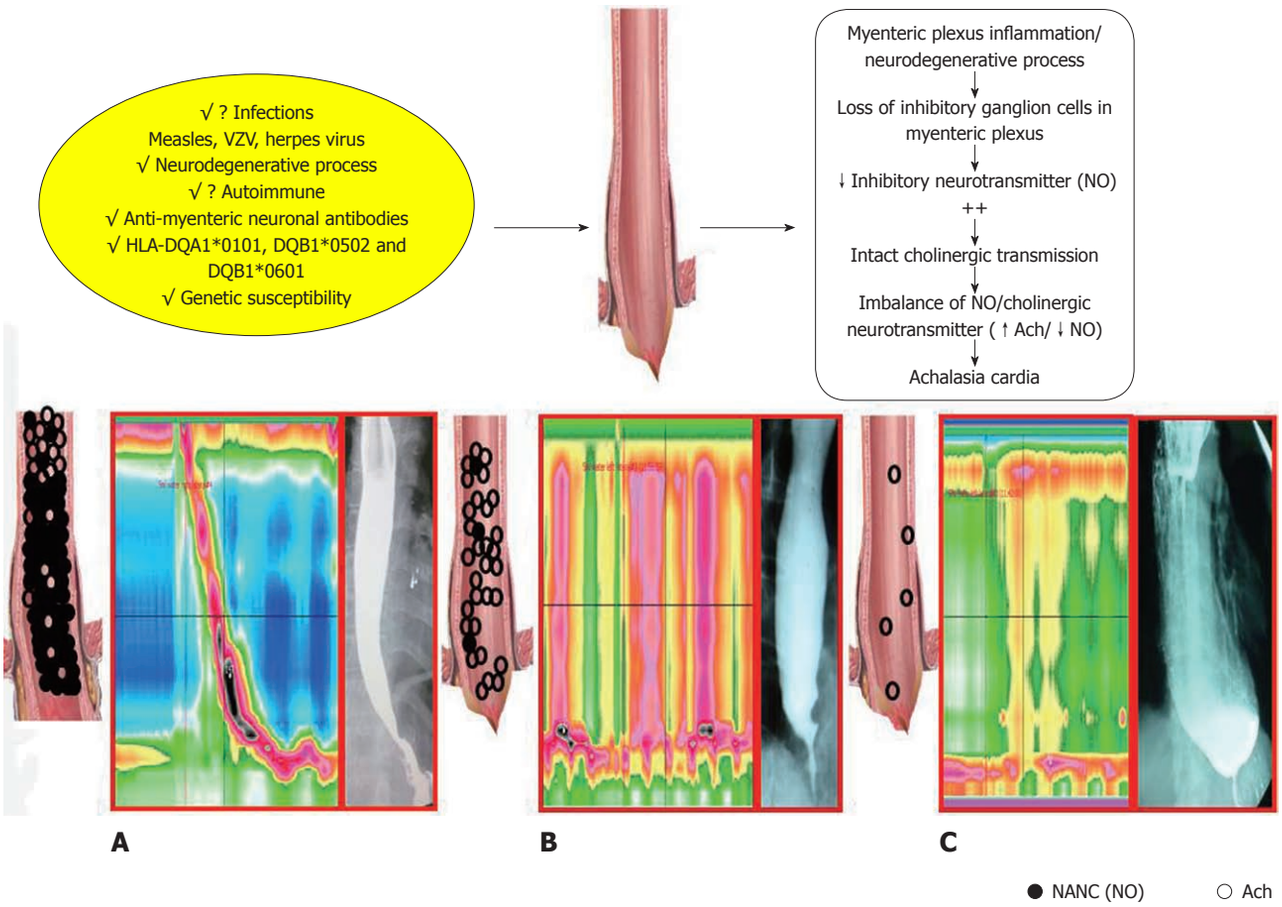

Figure 1 Schematic diagram outlining possible pathogenesis of achalasia cardia. A: Diagram showing distribution of nitric oxide (NO) and acetycholine (Ach) neurons in the esophagus with normal motility pattern and barium esophagogram; B: Loss of NO in esophagus in early stage of the disease resulting in high amplitude simultaneous contractions in body (called vigorous achalasia). In this stage esophagus is not dilated in barium esophagogram; C: Further degeneration of inhibitory and additional degeneration of excitatory neurons causes low amplitude simultaneous contractions in esophageal body (called classic achalasia). In this stage esophagus is dilated in barium esophagogram. NANC: Non-adrenergic, non-cholinergic.

to detect human herpes virus, MV and human papilloma virus sequences both in achalasia or control specimens[26]. Two studies proposed an association between MV and VZV infection in patients with achalasia[22,23]. However, VZV particles were found only in a third of achalasia patients[23]. Moreover, not all patients with VZV and MV infection develop achalasia. It has been hypothesized that most patients might have cleared virus or there could be sampling error. However, most of these data suggest that VZV may not be an important cause of achalasia.

Considering the above possibilities, a recent study tried to demonstrate infection in the absence of direct evidence of virus in esophageal tissue. Facco *et al*[27] reported oligoclonal selection of T cells in achalasia patients by flow cytometry and CDR3 length spectra typing analysis of lymphocytes. They also demonstrated increased proliferation of T cells and Th-1 type cytokine release in response to HSV-1 antigen. In conclusion, available evidence suggests that infection may not be a definite cause for esophageal achalasia. One strong piece of evidence in favor of infection in the pathogenesis of achalasia, however, is the fact that Chagas disease, caused by *Trypanosoma cruzi*, very closely mimics the pathophysiology of primary achalasia[28].

Immunological factors

An autoimmune etiology for achalasia has been considered because of the presence of neural inflammation in absence of conclusive evidence of infection. Studies have demonstrated inflammatory cell infiltrate of the myenteric plexus in 90%-100% of esophageal specimens from achalasia patients[29,30]. This hypothesis has been further supported by the presence of autoantibody in sera of patients with achalasia (Table 1) and an association with major histocompatibility complex class Ⅱ antigen (Table 2). Immune activation and inflammation is known to be associated with altered gastrointestinal motility due to neural dysfunction in the gut[31]. Taking the analogy of other gastrointestinal motility disorders such as post-infectious irritable bowel syndrome, intestinal pseudo-obstruction and ileus, it has been postulated that achalasia may have a similar pathophysiological basis[31]. However, more studies are needed on this issue.

Autoantibodies in achalasia: Several studies showed a higher prevalence of autoantibodies in achalasia patients compared to controls. Storch *et al*[32] suggested a role of autoimmunity by demonstrating a higher prevalence of anti-myenteric autoantibody in achalasia patients (64%)

Table 1 Studies comparing autoantibody levels between achalasia patients and control subjects

Authors	Tests	Autoantibody, *n*/N (%)		*P* value
		Achalasia	Control	
Storch *et al*[32]	Indirect immunofluorescence	37/58 (64)	4/54 (7) in healthy Absent in 12 of Hirschsprung's disease Absent in 12 esophageal cancer	< 0.0001
Verne *et al*[33]	Double-label Indirect immunofluorescence	7/18 (40)	Absent in 22 healthy Absent in 9 GERD	< 0.05
Ruiz-de-León *et al*[34]	Indirect immunofluorescence	50/92 (54.3)	3/40 (7.5)	< 0.001
Moses *et al*[36]	Immunohistochemistry	23/45 (51)	2/22 (9) in healthy control 8/16 (50) of GERD	< 0.001 NS

GERD: Gastroesophageal reflux disease; NS: Not significant.

Table 2 Studies showing association between achalasia and human leukocyte antigen

Authors	Allele	Allele frequency, *n*/N (%)		P_c value	Autoantibody in achalasia, *n*/N (%)		*P* value
		Achalasia	Control		AM positive	AM negative	
Ruiz-de-León *et al*[34]	DQA1*0103	27/92 (29.3)	40/275 (14.5)	< 0.02	22/50 (44)	5/42 (20)	< 0.02
	DQB1*0603	23/92 (25)	33/275 (12)	0.05	19/50 (38)	4/42 (10)	0.05
Latiano *et al*[40]	DQB1*0502	(10.2)	(4.1)	0.016	Antineuronal antibodies:		
	DQB1*0601	(5.93)	(0.51)	0.001	10/41 (24.4) in achalasia		
	DQA1	-	-	NS	None in controls		
					Both HLA risk allele and antibody: in one patient		
					None in controls		

AM: Anti-myenteric; HLA: Human leukocyte antigen; NS: Not significant.

compared to healthy controls (7%). They also showed absence of anti-myenteric autoantibody in Hirschsprung's disease as well as in esophageal cancer patients, and in 8%-9% of patients with peptic esophagitis and myasthenia gravis[32]. This hypothesis was further supported by subsequent studies[33,34].

Another interesting study demonstrated that patients with Chagasic achalasia more often had autoantibodies against muscarinic acetylcholine receptors [M(2) mAchR] as compared to patients with achalasia not resulting from Chagas disease[35].

Verne *et al*[33] tried to demonstrate the presence of regional and cellular specific antibody in achalasia patients. Staining of the esophageal and intestinal sections of rat with sera from achalasia patients showed binding of antibody to neurons in the esophageal as well as intestinal sections, though none of the sera of patients with gastroesophageal reflux disease (GERD) or controls showed staining[33]. Since idiopathic achalasia is primarily a disorder of esophageal smooth muscles, binding on intestinal sections may suggest non-specific binding of this antibody and hence, may not suggest a causal association.

A study by Moses *et al*[36] demonstrated a similar degree of immune-reactivity in the myenteric plexus of the esophagus and ileum of guinea-pig and mouse when immune-stained with sera of achalasia and GERD patients; however, both the groups had higher immune-reactivity when compared with normal individuals. Western blotting analysis failed to reveal specific myenteric neuronal proteins that could be targeted by antibodies in achalasia or GERD serum[35]. These observations do not support anti-neuronal antibodies to be causative in achalasia. These antibodies may perhaps be an epiphenomenon.

Human leukocyte antigen association: An association between achalasia and human leukocyte antigen (HLA) class Ⅱ histocompatibility antigens was proposed for the first time by Wong *et al*[37]. They performed HLA typing on 40 achalasia patients and found that Caucasians and black populations with DQw1 had 4.2 and 3.6 times higher risk of developing achalasia, respectively[37]. Subsequent studies[38,39] demonstrated associations between achalasia and HLA-DQA1*0101 allele, DQB1*0602 allele and DRB1*15 allele, and confirmed the findings reported by Wong *et al*[37]. Another study showed higher frequency of DQA1*0103, DQB1*0603 and DQA1*0103-DQB1*0603 heterodimer in patients with achalasia compared to controls. They also demonstrated that achalasia patients with DQA1*0103 and DQB1*0603 alleles had significantly higher prevalence of anti-myenteric antibody[34]. In contrast, Latiano *et al*[40] failed to show any correlation among HLA alleles and anti-neuronal antibodies. However, achalasia patients had higher frequency of DQB1*0502 and DQB1*0601 alleles. All these reports might suggest an autoimmune etiology of achalasia; however, not all patients with achalasia had predisposing HLA while not all people with the specific HLA had the disease.

In conclusion, all these data are not sufficient to con-

clude that achalasia is an autoimmune disease. To define a disease as autoimmune in nature, the disease should provide the following three features: (1) direct evidence based on transfer of disease by antibodies; (2) indirect evidence based on reproduction of autoimmune disease in an animal or genetic model; and (3) detection of autoreactive T cells in the target organ of disease[41]. To date, all the evidence to support an autoimmune etiology of achalasia has not been substantiated. More studies are needed on this issue.

Genetic factors: Some evidence supports a genetic basis for achalasia. The disease has been reported in monozygotic twins[42] and siblings[43-45]. A few reports described familial occurrence of achalasia cardia[46]. Studies showed genetic correlations of the *ALADIN* gene in achalasia associated with Allgrove syndrome[47,48]. Additionally, the disease has also been reported in association with other genetic diseases such as Down's syndrome and Parkinson disease[49,50]. The genetic basis for achalasia might have been underestimated, in contrast to that for Hirschsprung's disease, though both these enteric neuropathies have several features in common[51]. Hirschsprung's disease, like achalasia, is also known to have familial occurrence and the former has been reported quite commonly in different syndromes with a genetic basis. Both the conditions have altered motor function with loss of inhibitory innervation[51]. We wish to review some studies on genetic polymorphisms in certain genes in patients with achalasia cardia.

Nitric oxide synthase polymorphism: Nitric oxide is a necessary inhibitory neurotransmitter of the esophageal myenteric plexus involved in esophageal sphincter relaxation. Nitric oxide synthase (NOS) synthesizes nitric oxide from L-arginin. Reported studies showed that in patients with achalasic cardia, the LES has less NOS compared with controls[52,53]. At present, three different NOS isozymes, neuronal NOS (NOS1), inducible NOS (NOS2) and endothelial NOS (NOS3) have been reported. *NOS1*, *NOS2* and *NOS3* genes are located on human chromosomes 12q24.2, 17q11.2-q12 and 7q36, respectively. A microsatellite (CA repeat) polymorphism is found within the 3'-untranslated region of exon 29 of the *NOS1* gene. The *NOS2* gene has two biallelic polymorphisms in exons 16 and 22 representing C/T and G/A transitions, respectively, and *NOS3* shows a 27-bp variable number of tandem repeat polymorphisms in intron 4. Published studies have failed to explain a strong role for these functional polymorphisms in the susceptibility for achalasia[54,55].

***VIPR1* gene polymorphism:** The *VIPR1* gene is located on human chromosome 3p22[56]. Vasoactive intestinal peptide is a small neuropeptide, which acts as a neurotransmitter with anti-inflammatory properties; it is present in the myenteric plexus to modulate relaxation of the distal esophageal wall and LES[57-59]. Reported studies have suggested a role for this neuropeptide as chief element in the maintenance of neuro-endocrine immune system communication that activates signal transduction through specific receptors present on immune cells[60]. Receptor of vasoactive intestinal polypeptide (VIPR1) belongs to the secretin receptor family, a group of G-protein coupled receptors expressed by different immune cells, including T cells, macrophages and dendritic cells[56]. VIPR1 is present on myenteric neurons of the distal esophagus and LES; continued inflammation leads to the impairment of VIPR1 signaling, which alters the effect of VIP on myenteric neurons that progresses to ganglion cell loss and nerve fiber fibrosis[8,29,32-34,36,53]. A few further studies among patients with rheumatic diseases showed the receptor down-regulation of the *VIPR1* gene resulting in decreased signaling[61-64]. Paladini *et al*[64,65] reported five single nucleotide polymorphisms in the *VIPR1* gene in patients with achalasia cardia which included (rs421558) Intron-1, (rs437876) Intron-4, (rs417387) Intron-6, rs896 and rs9677 (3'UTR) and they found that the presence of A allele at (rs421558) Intron-1 and C allele at rs896 (3' UTR) was associated with a less efficient down-regulation of the receptor. Hence, AC haplotype may protect from the disease by down-regulation of VIPR1 receptor during inflammation.

Interleukin-23 receptor polymorphism: The interleukin-23 receptor (*IL-23R*) gene, located on chromosome Ip31, encodes for heterodimeric subunits of the IL-23R complex[66]. IL-23R is a type I trans-membrane protein expressed on Th1 derived T-cells which produce IL-17, designated as Th17 cells. Previous data support a role for Th17 cells and these cytokines in inflammatory and autoimmune processes[67]. Activated JAK-STAT signaling pathway by IL-23 binds with the IL-23R/IL-12β1 receptor, which influences the number of downstream immune responses[66]. Reported studies showed that IL-23R is associated with inflammatory as well as chronic autoimmune disorders[68-74]. In a single nucleotide polymorphism of the *IL-23R* gene, arginine replaces glutamine at codon 381. One study reported *IL-23R* coding variant 381Gln was a protective allele, whereas another study found this variant to be a risk factor. de León *et al*[75] reported coding variant 381Gln of *IL-23R* was significantly more common in patients with achalasia as compared with healthy controls. Hence, this 381Gln variant of *IL-23R* polymorphism could be a risk factor for achalasia cardia.

Protein tyrosine phosphatase non-receptor 22 gene polymorphism: The protein tyrosine phosphatase non-receptor 22 (*PTPN22*) gene is located on chromosome 1p13.3-p13, a region associated with autoimmune disease[75,76]. It encodes a lymphoid specific phosphatase known as Lyp. Lyp is an intracellular protein tyrosine phosphatase and an important down-regulator of T-cell activation[77]. The *PTPN22* gene exhibits a single nucleotide polymorphism at position 1858C/T, which leads to a replacement in codon 620 of Arg (R) to Trp (W). A study

showed that Lyp-W620 had more phosphatase activity, which could slow down T-cell signaling more effectively than Lyp-R620. In a different population it has already been reported that the *PTPN22* T allele is a risk factor for autoimmune diseases[78-83]. Santiago *et al*[84] reported that *PTPN22* C1858T polymorphism increased risk of achalasia in a Spanish population.

CONCLUSION

Achalasia is caused by loss of inhibitory ganglion in the myenteric plexus in the esophagus. Gradual progression of neuronal degeneration is associated with progression of the disease from vigorous to classic achalasia. Though several studies have attempted to explore initiating agents that may cause the disease, the exact factors responsible for the degeneration of ganglion cells in the myenteric plexus are poorly understood. The disease is likely to be multi-factorial involving host genetic factors, autoimmunity, and environmental factors such as infections. More studies are needed to explore the exact cause of this enigmatic disease.

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