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The relationship of recurrent aphthous stomatitis and *Helicobacter pylori*, cytokine gene polymorphism and cobalamin

STOMACH

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Original Article

ABSTRACT

Background/Aims: The aim of the present study was to investigate whether *Helicobacter pylori* causes or triggers recurrent aphthous stomatitis (RAS) through cytokine gene polymorphism and/or cobalamin deficiency.

Materials and Methods: Thirty-six patients with RAS and 130 patients without RAS were genotyped for IL-1β (–511C/T) and IL-6 (–174G/C) and evaluated for *H. pylori* infection and serum cobalamin level.

Results: The patient groups according to RAS had similar rates of *H. pylori* gastritis and interleukin genotypes/alleles, and there was a non-significant difference between serum cobalamin levels (p>0.05). RAS patients with *H. pylori* gastritis showed a higher frequency (51.9%) of GC IL-6 genotype than RAS patients without *H. pylori* gastritis (11.1%) (p=0.036). Non-GG genotype and C allele were increased in patients without RAS and with *H. pylori* gastritis (p<0.05). Patients with *H. pylori* gastritis showed a lower value of serum cobalamin without statistical significance, although this difference was more prominent in RAS patients (p=0.07).

Conclusion: The carriage of the C allele of IL-6 may lead a susceptibility to chronic gastric inflammation after contamination with *H. pylori*. If *H. pylori* infection is justified as a predisposing factor for RAS and its severity by further studies, we can speculate that subjects with genetic susceptibility to this infection may benefit from *H. pylori* eradication treatment with respect to RAS.

Keywords: Recurrent aphthous stomatitis, *H. pylori*, interleukin-6, interleukin-1β, polymorphism, serum cobalamin

INTRODUCTION

The term recurrent aphthous stomatitis (RAS) refers to the most common inflammatory ulcerative disorder of the oral mucosa (1). RAS is characterized by painful superficial ulcers on non-keratinized or poorly keratinized mucosa and is generally localized (2). The etiopathogenesis of RAS is unclear, although many possible predisposing factors have been implicated such as trauma, emotional stress, hormonal state, food hypersensitivity, viruses, bacteria, and immune dysregulation (3). It has also been reported that type 1 helper (Th1) genes are locally over expressed and cytokines such as interleukin-2 (IL-2), tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6) are systemically produced by circulating mononuclear cells. Interleukin-10 (IL-10) messenger RNA (mRNA) levels were reported to be decreased, which suggests the immune system cannot effectively suppress the inflammatory reaction against the oral mucosa (4,5). Previous studies have reported that increased cell-mediated immune responses against certain areas of the oral mucosa secondary to an abnormally regulated cytokine cascade leads to ulceration (5). RAS etiopathogenesis may also include genetic factors and RAS patients have an increased prevalence of HLA-B12 and Behçet's disease, which is a disorder characterized by recurrent aphthous ulcers and is associated with increased HLA-B5 levels (6). It has been reported that interleukin-1β and IL-6 gene polymorphisms are associated with a significant risk for the development of RAS. However, associations between specific IL-10 or interleukin-12 gene polymorphism and RAS susceptibility were not demonstrated in prior studies (7-9). Studies

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have shown that a G allele at the −174 position is associated with a higher IL-6 production than a C allele at the same position (10). Similar relationships between IL-1β −511 genotype and IL-1β production were also suggested (11).

Histological similarities between gastric and oral ulceration suggest a possible association between *Helicobacter pylori* and RAS. *H. pylori* infections were identified in the oral tissue by polymerase chain reaction (PCR) in a small proportion of RAS cases (12). Furthermore, *H. pylori* eradication may lead to fewer recurrences and longer RAS disease-free intervals (13,14).

If there is a causal relationship between *H. pylori* and RAS, then there must be additional factors explaining how this microorganism leads to RAS development in some patients. The cytokine production pattern during *H. pylori* infection may have a role in RAS outcome. *H. pylori*-associated pathologies such as peptic ulcer and gastric cancer may be related to polymorphisms of cytokines such as IL-1β, IL-10, interferon-γ, or TNF-α (15). RAS patients with a particular cytokine gene polymorphism may have more frequent problems of RAS when they are infected with *H. pylori*.

Another defined abnormality in patients with RAS is the low level of serum cobalamin (16,17). RAS is improved by replacement therapies and patients with RAS have a higher rate of cobalamin deficiency, suggesting such a relationship. *H. pylori* has been proposed as a causative role for low levels of serum cobalamin (18,19). It is possible that *H. pylori* infection can trigger RAS in some patients through cobalamin deficiency. The aim of the present study was to investigate whether *H. pylori* infection causes RAS through cytokine gene polymorphisms and/or cobalamin deficiency.

MATERIALS AND METHODS

This study was performed at the outpatient clinics of the Rheumatology and Gastroenterology departments of our institution. After the approval of the study by the Başkent University Ethics Committee, it was conducted in accordance with the principles of the Declaration of Helsinki and the recommendation of the ethics committee. All subjects gave a written informed consent before blood sample collection.

Subjects and sample collection

Thirty-six patients with RAS and 130 patients without RAS were enrolled into the study. All patients underwent a detailed review of past history and physical examination. All RAS lesions that occurred within the last 6 months were recorded. Diagnosis of RAS was made on clinical grounds by a rheumatologist with sufficient experience for the management of RAS. The inclusion criterion for the patients was to have at least three minor RAS attacks in the last year. The ulcers are round or oval lesions with a diameter of 1 cm, a necrotic center, and erythematous borders. History and clinical signs still remain as the basis of the diagnosis of RAS as no disease-specific laboratory tests are yet available (20). All patients with systemic diseases associated with oral aphthous ulcers were excluded. In addition, patients with the following criteria were excluded: 1) pregnancy; 2) using medications with a potential to cause oral ulcers or interact with *H. pylori* infection (antibiotics, proton pump inhibitors, H_2 receptor antagonists, bismuth derivatives, nonsteroidal anti-inflammatory drugs, chemotherapeutic medications, or vitamin supplements) for the last 2 months before study entry; 3) starting or stopping smoking after being enrolled in the study; 4) history of systemic disease with a potential to cause oral ulceration (Behçet's disease, coeliac disease, Crohn's disease, ulcerative colitis, or AIDS); 5) using systemic or topical steroids, immunomodulatory drugs or cytotoxic drugs, e.g. Azathioprine, Cyclosporin, Levamisole, Thalidomide, Pentoxifylline, Prednisolone, 5-amino salicylic acid, or Colchicine; (6) an ulceration pattern suggesting major RAS (A rare, severe form of RAS, sometimes referred to as periadenitis mucosa necrotica recurrens. It is characterized by oval ulcers that may have a diameter of greater than 1 cm) or herpetiform ulceration with characteristic multiple, recurrent bouts of diffuse, small, painful ulcers, each with a diameter of 2–3 mm (21).

An endoscopic biopsy was taken from each patient with the help of a Pentax EG-2985 video endoscope. Biopsies were collected from two locations of the antral and body regions of the stomach and the tissue was prepared with Giemsa stain. The tissues were then examined for *H. pylori* infection.

Chemiluminescent Micro particle Immunoassay (CMIA) commercial kit (Abbott Laboratories, Abbott Park, IL, USA) was used to measure serum cobalamin levels in an Architech analyzer. A reference range of 179–1162 pg/mL was used for cobalamin.

Genotyping

Each patient collected a ten mL venous blood sample that was put in the standardized tubes containing 0.072 mL 7.5% ethylenediaminetetraacetic acid solution. Genomic DNA was isolated and kept at −80°C for subsequent molecular analysis. Each study subject provided a peripheral blood sample, and then a PCR template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to extract genomic DNA from circulating leukocytes. The genotypes of IL-1β (−511C/T) and IL-6 (−174G/C) were determined by restriction fragment length polymorphism analyses according to the slightly modified procedures previously described (22-25). The IL-1β (−511C/T) polymorphism was identified after digestion with AvaI, which yielded 305-bp bands in the presence of allele C and 190- and 115-bp bands in the presence of allele T. The −174G/C polymorphism of the IL-6 gene was identified after digestion with the restriction enzyme NlaIII, which yielded 121-, 109-, and 75-bp bands in the presence of allele C and 230 and 75-bp bands in the presence of allele G.

Statistical analysis

Statistical Package for the Social Sciences for Windows, version 19.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses. The

comparisons of age and serum cobalamin levels of patients were analyzed using the Mann–Whitney U test. The statistical significance of differences between groups for alleles and genotypes was determined using the chi-squared test. A p-value of less than 0.05 was accepted as significant. The distributions of genotypes for the two types of polymorphisms were compared with those expected from the Hardy–Weinberg equilibrium.

RESULTS

Patients with RAS were younger than patients without RAS (p<0.05). These two patient groups had similar rates of *H. pylori* gastritis and interleukin genotypes/alleles, and there was no significant difference between serum cobalamin levels, as shown in Table 1.

The distributions of both interleukin genotypes were not different from the expected Hardy–Weinberg equilibrium. After stratifying patients according to RAS and *H. pylori* gastritis, RAS patients with *H. pylori* gastritis showed a higher frequency (51.9%) of GC IL-6 genotype than RAS patients without *H. pylori* gastritis (11.1%) (p=0.036). When we analyzed the same comparison in patients without RAS using GG and non-GG genotypes (CC or GC), the difference between patients with *H. pylori* and without *H. pylori* was also significant (p<0.045). The frequency of the C allele was higher in patients with *H. pylori* gastritis and was statistically significant in patients without RAS. The GG genotype was more prominent in *H. pylori* negative groups independent of their RAS status, as shown in Table 2. Patients with *H. pylori* gastritis showed lower values of serum cobalamin; however, the values were not statistically significant. The difference was more prominent in RAS patients (p=0.07), as shown in Table 2.

Table 1. Demographic characteristics and cobalamin and cytokine gene polymorphism (IL-6 and IL-1β) results of patients according to the RAS and *H. pylori* status

	Patients without RAS (n=130)	Patients with RAS (n=36)	p value
Age, median (range)	50 (18–84)	40 (18–70)	0.004
Gender (male/female)	59/71	15/21	0.71
H. pylori (+) gastritis, n (%)	87 (66.9)	27 (75.0)	0.42
Serum cobalamin level* Median (range)	214 (60–926)	188 (109–485)	0.08
IL-6, n (%)			
Genotypes			
GG	78 (60.0)	21 (58.3)	0.21
CC	9 (6.9)	0 (0.0)	
GC	43 (33.1)	15 (41.7)	
Alleles			
G	199 (76.5)	57 (79.2)	0.75
C	61 (23.5)	15 (20.8)	
IL-1β, n (%)			
Genotypes			
CC	39 (30.0)	8 (22.2)	0.54
TC	67 (51.5)	19 (52.8)	
TT	24 (18.5)	9 (25.0)	
Alleles			
C	145 (55.8)	35 (48.6)	0.28
T	115 (44.2)	37 (51.4)	

*: pg/mL
RAS: recurrent aphthous stomatitis
IL: interleukin

DISCUSSION

The aim of the study was to evaluate the role of IL-1β and IL-6 polymorphisms and cobalamin deficiency in the development of RAS in cases of *H. pylori* infection. The study was conducted and designed with the acceptance of a positive relationship between *H. pylori* infection and RAS reported by recent studies. We hypothesized that a genetic factor and/or cobalamin deficiency can be decisive or facilitative in the formation of recurrent oral aphthous ulcers in subjects with *H. pylori*.

There are several studies and observations supporting that IL-1β plays a pivotal role in RAS and other chronic diseases The polymorphisms of the IL-1β gene at −511 and at +3954 were found more frequently in patients with RAS (25), and these genotypes were associated with an increased production of IL-1β after stimulation. The increased expression of IL-1β cDNA in apthous lesions supports the suggestions of epidemiologic studies (26). However, in our study, we did not find any correlation between IL-1β polymorphism and RAS development. The absence of any correlation in our study group may be due to heterogeneity and genetic variation of the population. Another explanation is that the amount of IL-1β production may be regulated mainly by the IL-1RA gene, which is one of the genes in the IL-1 complex that has an allelic association with the IL-1β gene (27). Thus, we may have falsely evaluated IL-1β gene polymorphisms instead of IL-1RA genes in our study subjects.

We did not find significant differences in IL-6 genotypes and alleles between patients with RAS and those without RAS. However, the non-GG genotype (GC or CC) was more frequent in *H. pylori* positive patients with RAS and without RAS. This result suggested that the C allele of the IL-6 gene is a factor in the pathogenesis of *H. pylori* infection. The presence of the C allele may increase the susceptibility to chronic gastric inflammation after *H. pylori* infection. If *H. pylori* infection has any role in the development or severity of RAS by other mechanisms that we have not investigated in this study, then patients with genetic susceptibility to *H. pylori* and IL-6 polymorphisms should be evaluated for this infection.

Another possible relationship between *H. pylori* infections and RAS may depend on cobalamin deficiency secondary to

Table 2. Demographic characteristics and cobalamin and cytokine gene polymorphism (IL-6 and IL-1β) results of patients according to the RAS and *H. pylori* status

	Patients without RAS (n=130)			Patients with RAS (n=36)		
	H. pylori gastritis			*H. pylori* gastritis		
	Positive (n=87)	Negative (n=43)	p value	Positive (n=27)	Negative (n=9)	p value
Age, median (range)	50 (19–84)	51 (18–83)	0.42	41 (18–70)	36 (19–48)	0.17
Gender male/female	43/48	23/20	0.99	17/10	4/5	0.44
Serum cobalamin level* median (range)	200 (60–926)	246 (60–784)	0.29	174 (109–317)	228 (116–485)	0.07
IL-6, n (%)						
Genotypes						
GG	47 (54.0)	31 (72.1)	0.099	13 (48.1)	8 (88.9)	0.036
CC	8 (9.2)	1 (2.3)		-	-	
GC	32 (36.8)	11 (25.6)		14 (51.9)	1 (11.1)	
Non-GG (GC or CC)	40 (46)	22 (27.9)	0.045			
Alleles						
G	126 (72.4)	73 (84.9)	0.029	40 (74.1)	17 (94.4)	0.095
C	48 (27.6)	13 (15.1)		14 (25.9)	1 (5.6)	
IL-1β, n (%)						
Genotypes						
CC	28 (32.2)	11 (25.6)	0.713	5 (18.5)	3 (33.3)	0.650
TC	44 (50.6)	23 (53.5)		15 (55.6)	4 (44.4)	
TT	15 (17.2)	9 (20.9)		7 (25.9)	2 (22.2)	
Alleles						
C	100 (57.5)	45 (52.3)	0.507	25 (46.3)	10 (55.6)	0.590
T	74 (42.5)	41 (47.7)		29 (53.7)	8 (44.4)	

*: pg/mL
RAS: recurrent aphthous stomatitis
IL: interleukin

H. pylori gastritis, which has been reported by several studies (19,28,29). Low serum cobalamin level and *H. Pylori* gastritis have been reported to be strongly correlated via mechanisms suggested or reported by authors (18,19). In addition, several studies of nutritional deficiencies in patients with RAS have reported low levels of serum cobalamin (30,31). Furthermore, vitamin replacement in patients improves their RAS-related symptoms (32,33). Kozlak et al. (34) demonstrated that patients with RAS were more likely to have lower dietary intakes of cobalamin and folate than the control group. Apart from these epidemiological studies, the association between cobalamin deficiency and RAS has not been fully elucidated. Cobalamin is an important co-enzyme for a number of metabolic reactions including protein synthesis (35). Thus, cobalamin deficiency may disrupt healing process and cause ulceration. In our study, patients with *H. pylori* gastritis represented a lower value of serum cobalamin without statistical significance, being more prominent in RAS patients. To our opinion, genetically and environmentally determined tendency to RAS may be exacerbated by cobalamin deficiency that is sometimes caused by *H. pylori* gastritis.

In conclusion, we can speculate that the treatment for *H. pylori* infection in RAS patients with genetic susceptibility to chronic *H. pylori* gastritis should decrease RAS symptoms by some undefined mechanisms and improvement in serum cobalamin level can be effective.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Başkent University Faculty of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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REFERENCES

1. Robinson ND, Guitart J. Recalcitrant, recurrent aphthous stomatitis treated with etanercept) Arch Dermatol 2003; 139: 1259-63. **[CrossRef]**
2. Jurge S, Kuffer R, Scully C, Porter SR. Mucosal Diseases Series, Number VI Recurrent aphthous stomatitis. Oral Dis 2006; 12: 1-21. **[CrossRef]**
3. Embil JA, Stephens RG, Manuel FR. Prevalence of recurrent herpes labialis and aphthous ulcers among young adults on six continents. Can Med Assoc J 1975; 113: 627-30.
4. Borra RC, Andrade PM, Silvaet ID, al. The Th1/Th2 immune-typeresponse of the recurrent aphthous ulceration analyzed by cDNA microarray J Oral Pathol Med 2004; 33: 140-6. **[CrossRef]**
5. Buño IJ, Huff JC, Weston WL, Cook DT, Brice SL. Elevated levels of interferon gamma, tumor necrosis factor alpha, interleukins 2, 4 and 5, but not interleukin 10, are present in recurrent aphthous stomatitis Arch Dermatol 1998; 134: 827-31.
6. Riggio MP, Lennon A, Wray D. Detection of Helicobacter pylori DNA in recurrent aphthous stomatitis tissue by PCR. J Oral Pathol Med 2000; 29: 507-13. **[CrossRef]**
7. Bazrafshani MR, Hajeer AH, Ollier WE, Thornhill MH. IL-1β and IL-6 gene polymorphisms encode significant risk for the development of recurrent aphthous stomatitis (RAS). Genes Immun 2002; 3: 302-5. **[CrossRef]**
8. Guimarães AL, de Sá AR, Victória JM, et al. Association of interleukin-1 beta polymorphism with recurrent aphthous stomatitis in Brazilianin dividuals. Oral Dis 2006; 12: 580-3. **[CrossRef]**
9. Bazrafshani MR, Hajeer AH, Ollier WE, Thornhill MH. Polymorphisms in the IL-10 and IL-12 gene cluster and risk of developing recurrent aphthous stomatitis. Oral Dis 2003; 9: 287-91. **[CrossRef]**
10. Fishman D, Faulds G, Jeffery R et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic onset juvenile chronic arthritis. J Clin Invest 1998; 102: 1369-76. **[CrossRef]**
11. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqIpolymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992; 22: 396-402. **[CrossRef]**
12. Riggio MP, Lennon A, Wray D. Detection of Helicobacter pylori DNA in recurrent aphthous stomatitis tissue by PCR. J Oral Pathol Med 2000; 29: 507-13. **[CrossRef]**
13. Karaca S, Seyhan M, Şenol M, Harputluoğlu MM, Özcan A. The effect of gastric Helicobacter pylori eradication on recurrent aphthous stomatitis. Int J Dermatol 2008; 47: 615-7. **[CrossRef]**
14. Taş DA, Yakar T, Sakalli H, Serin E. Impact of Helicobacter pylori on the clinical course of recurrent aphthous stomatitis. J Oral Pathol Med 2013; 42: 89-94. **[CrossRef]**
15. Basso D, Plebani M. H. pylori infection: bacterial virulence factors and cytokine gene polymorphisms as determinants of infection outcome. Crit Rev Clin Lab Sci 2004; 41: 313-37. **[CrossRef]**
16. Barnadas MA, Remacha A, Condomines J, de Moragas JM. Hematologic deficiencies in patients with recurrent oral aphthae. Med Clin 1997; 109: 85-7.
17. Baccaglini L, Lalla RV, Bruce AJ, et al. Urban legends: recurrent aphthous stomatitis. Oral Dis 2011; 17: 755-70. **[CrossRef]**
18. Carmel R, Aurangzeb I, Qian D. Associations of food-cobalamin malabsorption with ethnic origin, age, Helicobacter pylori infection, and serum markers of gastritis. Am J Gastroenterol 2001; 96: 63-70. **[CrossRef]**
19. Serin E, Gümürdülü Y, Ozer B, Kayaselçuk F, Yilmaz U, Koçak R. Impact of Helicobacter pylori on the development of vitamin B12 deficiency in the absence of gastric atrophy. Helicobacter 2002; 7: 337-41. **[CrossRef]**
20. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: a consensus approach. J Am Dent Assoc 2003; 134: 200-7. **[CrossRef]**
21. Preeti L, Magesh K, Rajkumar K, Karthik R. Recurrent aphthous stomatitis. J Oral Maxillofac Pathol 2011; 15: 252-6. **[CrossRef]**
22. Erbek SS, Yurtcu E, Erbek S, Atac FB, Sahin FI, Cakmak O. Proinflammatory cytokine single nucleotide polymorphisms in nasal polyposis. Arch Otolaryngol Head Neck Surg 2007; 133: 705-9. **[CrossRef]**
23. Yılmaz Y, Verdi H, Taneri A, et al. Maternal-fetal proinflammatory cytokine gene polymorphism and preterm birth. DNA Cell Biol 2012; 31: 92-7. **[CrossRef]**
24. Pooja S, Chaudhary P, Nayak LV, et al. Polymorphic variations in IL-1β, IL-6 and IL-10 genes, their circulating serum levels and breast cancer risk in Indian women. Cytokine 2012; 60: 122-8. **[CrossRef]**
25. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J.A TaqI polymorphism in the human interleukin-1 beta (IL-1beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992; 22: 396-402. **[CrossRef]**
26. Bazrafshani MR1, Hajeer AH, Ollier WE, Thornhill MH. Thornhill IL-1β and IL-6 gene polymorphisms encode significant risk for the development of recurrent aphthous stomatitis (RAS). Genes Immun 2002; 3: 302-5. **[CrossRef]**
27. Santtila S, Savinainen K, Hurme M. Presence of theIL-1RAallele2 (IL1RN*2) is associated with enhanced IL-1betaproduction in vitro. Scand J Immunol 1998; 47: 195-8. **[CrossRef]**
28. Carmel R, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and food-cobalamin malabsorption. Dig Dis Sci 1994; 39: 309-14. **[CrossRef]**
29. Kaptan K, Beyan C, Ural AU, et al. Helicobacter pylori-Is it a novel causative agent in vitamin B12 deficiency? Arch Intern Med 2000; 160: 1349-53. **[CrossRef]**
30. Weusten BL, van de Wiel A. Aphthous ulcers and vitamin B12 deficiency. Neth J Med 1998; 53: 172-5. **[CrossRef]**
31. Piskin S, Sayan C, Durukan N, Senol M. Serum iron, ferritin, folic-acid, and vitamin B12 levels in recurrent aphthous stomatitis. J Eur Acad Dermatol Venereol 2002; 16: 66-7. **[CrossRef]**
32. Wray D, Ferguson MM, Mason DK, Hutcheon AW, Dagg JH. Recurrent aphthae: treatment with vitamin B12, folic acid, and iron. Br Med J 1975; 2: 490-3. **[CrossRef]**
33. Nolan A, McIntosh WB, Allam BF, Lamey PJ. Recurrent aphthous ulceration: vitamin B1, B2 and B6 status and response to replacement therapy. J Oral Pathol Med 1991; 20: 389-91. **[CrossRef]**
34. Kozlak ST, Walsh SJ, Lalla RV. Reduced dietary intake of vitamin B12 and folate in patients with recurrent aphthous stomatitis. J Oral Pathol Med 2010; 39: 420-3. **[CrossRef]**
35. Green R, Miller JW. Vitamin B12. In: Zemplini J, Rucker RB, Suttie JW, McCormick DB editors. Handbook of vitamins. 2nd ed. Boca Raton, FL: CRC Press; 2007. 413-57.