Interleukin-1 and tumor necrosis factor-α gene polymorphisms in Turkish patients with localized aggressive periodontitis

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(Received 3 September 2007 and accepted 1 April 2008)

Abstract: Localized aggressive periodontitis (LAgP) is a complex multifactorial periodontal disease to which genetic factors are thought to predispose individuals. Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) are potent immunomodulators and proinflammatory cytokines that have been implicated in the pathogenesis of autoimmune and infectious diseases and proposed to be risk factors for LAgP. Our aim was to investigate IL-1α+4845, IL-1β+3954, and TNF-α-308 gene polymorphisms in Turkish LAgP patients. We genotyped 31 LAgP patients and 31 healthy controls for IL-1α+4845, IL-1β+3954, and TNF-α-308 using standard PCR amplification followed by restriction enzyme digestion and gel electrophoresis. Higher prevalence of heterozygosity for IL-1α+4845 was found in cases (65%) when compared to controls (35%) (P < 0.05). While homozygous allele 1 of IL-1β+3954 was the most frequent genotype in cases (62%), no controls were homozygous for this allele (P < 0.001). Homozygous allele 1 was the most common TNF-α genotype in both groups, however no significant difference in TNF-α genotypes was found between groups. In conclusion, in this Turkish population, susceptibility to LAgP is increased by heterozygosity for allele 1 of IL-1α+4845 or homozygosity for allele 1 of IL-1β+3954. Moreover, IL-1 gene polymorphisms appear to have a role in susceptibility to LAgP, and the above-mentioned genotypes could be an important risk factor for LAgP in the Turkish population. (J. Oral Sci. 50, 151-159, 2008)

Keywords: localized aggressive periodontitis; interleukin-1α; interleukin-1β; tumor necrosis factor-α; genetic polymorphisms.

Introduction

Localized aggressive periodontitis (LAgP) is a complex, multifactorial, destructive inflammatory periodontal disease. Progression and severity of the disease depend on interacting risk factors such as immunological, microbiological, environmental, and genetic factors, as well as age, sex, and race (1-4). Some patients are genetically predisposed to LAgP; they have immunological abnormalities which are thought to be under genetic control. However, the relationship between inflammatory changes and genetic factors remains unclear.

Cytokines play an important role in numerous biological activities including proliferation, development, homeostasis, regeneration, repair, and inflammation (5). They are pleiotropic, taking part in and triggering inflammatory cascades and systems. Periodontal tissue cells stimulated by periodontal disease-associated bacteria secrete proinflammatory cytokines, such as interleukin (IL)-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, which play key roles in the pathogenesis of periodontal diseases. There is a positive correlation between existence
and activity of periodontal diseases and tissue cytokine levels (6,7). The proinflammatory cytokines IL-1 and TNF-α are considered major mediators of periodontal inflammation (8).

IL-1 (which has 2 main forms, IL-1α and IL-1β) is the prototypic “multifunctional” cytokine. IL-1 affects nearly every cell type, often in concert with other cytokines or small mediator molecules. IL-1 is the margin between clinical benefit and unacceptable toxicity in human. The varied biologic properties of IL-1 result from its effects on the expression of various genes that regulate the production of cytokines such as TNF-α, IL-2, IL-3, and IL-6 (8). IL-1 has been implicated in the pathogenesis and clinical course of periodontal diseases because of its multiple proinflammatory properties (9,10). It is a key mediator of inflammation and modulates extracellular matrix components, enhances bone resorption in the periodontal tissues, stimulates fibroblasts and other nucleated cells to produce matrix metalloproteinase, activates plasminogen, and triggers prostaglandin synthesis (5,9-12). IL-1 also strongly stimulates connective tissue catabolism, activates immunocytes, and regulates adhesion molecules that facilitate migration of leukocytes into tissues (12).

IL-1 family genes are located in a cluster on human chromosome 2q13 (13). A specific genotype in the IL-1 cluster that includes a specific locus is associated with increased IL-1 production and increased susceptibility to severe periodontitis (13).

TNF-α is another potent immunomodulator and proinflammatory cytokine that has been implicated in the pathogenesis of autoimmune and infectious disease. TNF-α induces the secretion of collagenase by fibroblasts, stimulates resorption of cartilage and bone, and has been implicated in the destruction of periodontal tissue in periodontitis (5,14,15). The TNF-α gene lies within the class III region of the major histocompatibility complex on the short arm of human chromosome 6. There is a base-transition polymorphism (a G to A polymorphism) at the -308 position of the TNF-α promoter region (16).

Higher production of IL-1 and TNF has been associated with enhanced response to infection, in which local induction of these cytokines facilitates elimination of the microbial invasion. TNF-α acts synergistically with IL-1.

Genetic markers have come to attention because of the genetic nature of AgP. In 1997, Kornman et al. noted the association of periodontal diseases and cytokine gene polymorphisms (10). There is evidence for an association between certain cytokine gene polymorphisms and human diseases that involve an inflammatory pathogenesis. Cytokine polymorphisms may influence the level of cytokine secretion and may explain the individual differences in the cytokine responses to bacterial stimuli. Moreover, allelic variation in genes for cytokines and for factors regulating their expression may create phenotypic differences in cytokine responses between individuals (17).

Since one prominent feature of periodontal disease is resorption of alveolar bone, particular attention has been paid to the roles of IL-1α, IL-1β, IL-6, IL-8, and TNF-α in pathogenesis, due to their enhancement of bone resorption. While several studies using different methods have been performed to investigate the possible association of IL-1 (18-21) and TNF-α (22-26) with gene polymorphisms linked to AgP in different ethnicities, the literature contains no data concerning the association of IL-1α+4845, IL-1β+3954, and TNFα-308 polymorphisms in Turkish LAgP patients.

In the present study we therefore aimed to determine whether IL-1α+4845, IL-1β+3954, and TNFα-308 are associated with gene polymorphisms in Turkish patients with LAgP.

### Materials and Methods

#### Clinical Procedures

All subjects were of Turkish Caucasian heritage and both LAgP patients and control individuals were free of systemic diseases. The study protocol was approved by the Ethics Committee of Ankara University, Faculty of Dentistry. Subjects participated in the study after providing informed consent and being advised about their disease. Evaluation of each participant consisted of personal and family medical history, full-mouth periapical radiographs, and dental examinations. Exclusion criteria were as follows: history of hepatitis, HIV infection, or diabetes; requirement for antibiotic prophylaxis; pregnancy or lactation; and long-term usage of anti-inflammatory drugs. Since cigarette smoking is a severe risk factor for periodontal disease, smokers were also excluded.

For LAgP patients, measurements of probing depth (PD), clinical attachment loss (CAL), and bleeding on probing (BOP) were made at six sites (mesiobuccal, buccal, distobuccal, mesiolingual/palatal, palatal/lingual, and distopalatal/lingual) for each tooth using a Williams Periodontal Probe (Carl Martin, Solingen, Germany). Gingival index (GI) and plaque index (PI) were recorded at four sites (mesiobuccal, buccal, distobuccal, and lingual/palatal). LAgP was defined as at least 2 teeth were affected – they could be either first molars or incisors with clinical attachment loss ≥ 4 mm. No local irritation caused by attachment loss was visible at these sites.
Thirty-one Ankara University Dental Faculty 5th grade students were recruited as controls, since they were aware of their medical histories. For controls, GI, PI, PD, and BOP were recorded at four sites (mesiobuccal, buccal, distobuccal, and lingual/palatal). Individuals who had CAL were excluded.

Sample collection and extraction of DNA
Venepuncture was performed to collect 9 ml peripheral blood from each subject into tubes containing 1 ml ethylenedinitrilotetraacetic acid (EDTA). Genomic DNA was extracted from whole blood using phenol/chloroform extraction and ethanol precipitation procedures. DNA was stored at +4°C until genotyping. DNA samples were subjected to polymerase chain reaction (PCR) using the primers described in the next section. PCR amplifications were performed in 33.2 µl volume containing 3 µl DNA. The resulting products were visualized by performing electrophoresis through a 3.5% agarose gel for IL-1α+4845 and TNFα-308, and through a 3% agarose gel for IL-1β+3954, staining with ethidium bromide; and photographing on an ultraviolet light transilluminator.

Analysis of IL-1α+4845 polymorphism
The primers 5’ ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA 3’ and 5’ AAT GAA AGG AGG GGA TGA CAG AAA TGT 3’ were used. The 153 bp region of the IL-1α+4845 gene was amplified for 45 cycles using the thermocycler, with each cycle consisting of 94°C for 1 min, 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and 72°C for 5 min. DNA was digested with Fnu4HI restriction endonuclease (New England Biolabs, Beverly, MA, USA) at 37°C. The resulting products of 124 bp + 29 bp (homozygous allele 1), 153 bp (homozygous allele 2), and 153 bp, 124 bp, and 29 bp (heterozygous) were diagnostic.

Analysis of IL-1β+3954 polymorphism
IL-1β+3954 genotypes were determined by PCR using the primers 5’ TC AGG TGT CCT CGA AGA AAT CAA A 3’ and 5’ GCT TTT TTG CTG TGA GTC CGG 3’. The IL-1β+3954 gene was amplified for 35 cycles using PCR, with each cycle consisting of 95°C for 2 min, 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min. DNA was digested with Taq I restriction endonuclease (New England Biolabs, Beverly, MA, USA) at 37°C. The resulting products of 97 bp + 85 bp (homozygous allele 1), 182 bp (homozygous allele 2), and 182 bp, 97 bp, and 85 bp (heterozygous) were diagnostic.

Analysis of TNFα-308 polymorphism
TNFα-308 genotypes were similarly determined by PCR using the primers 5’ TC AGG TGT CCT CGA AGA AAT CAA A 3’ and 5’ GCT TTT TTG CTG TGA GTC CGG 3’. The gene was amplified for 35 cycles, cycling at 94°C for 3 min, 60°C for 1 min, 72°C for 1 min, and 60°C for 1 min. The products were digested with NcoI (New England Biolabs, Beverly, MA, USA) at 37°C. TNFα-308 PCR products of 87 bp and 20 bp (homozygous allele 1), 107 bp (homozygous allele 2), and 107 bp, 87 bp, and 20 bp (heterozygous) were diagnostic.

Statistical Analysis
Data analyses were performed using a computer program (SPSS 10.0 for Windows, Chicago, IL, USA). The frequency of individuals who carried allele 1 and allele 2 and the frequency of heterozygosity and homozygosity for each genetic polymorphism was determined and compared between the groups using χ² test. The strength of the associations were determined using odds ratio (OR) calculations and 95% confidence intervals (CI).

The relationships between IL-1α, IL-1β, and TNF-α genetic polymorphisms (homozygous allele 1, homozygous allele 2, and heterozygous) were determined by the Kruskal-Wallis Test.

| Table 1 Demographic and clinical data of LAgP and control individuals |
|-------------------------------------------------|----------|----------|
| Female*                                         | 29 (93.5)| 30 (96.8) |
| Male*                                          | 2 (6.5)  | 1 (3.2)  |
| Mean age**                                      | 16-34 (25.32) | 18-24 (20.70) |
| Parental consanguinity*                        | 7 (22.6) | 0 (0)    |
| PD (mm) †                                      | 3.71 ± 0.64 | 1.85 ± 0.27 | *P < 0.001 |
| CAL (mm) †                                     | 3.20 ± 0.89 | N/A      |
| BOP (%) †                                      | 47.90 ± 20.33 | 14.87 ± 11.82 | *P < 0.001 |

* Numbers of each individuals and percentages in parenthesis
** Range of age and mean age in parenthesis
† Values are mean ± SD; PD, probing depth; CAL, clinic attachment loss; BOP, bleeding on probing.
Results

Thirty-one Turkish LAgP patients and 31 Turkish control individuals were recruited. Mean PD and mean BOP differed significantly between LAgP patients and controls ($P < 0.001$). Demographic and clinical data are shown in Table 1. Figure 1 shows mean CAL of LAgP patients. Mean CAL for molars in the LAgP group was significantly higher than that for premolars and incisors ($P < 0.001$).

While the prevalence of heterozygous alleles for IL-1α (1,2) was higher in the LAgP group (65%), the prevalence of IL-1α homozygous allele 1 (1,1) was higher in control individuals (65%). This difference was statistically significant ($\chi^2 = 8.785, P < 0.05$). In the LAgP group, the prevalence of IL-1α homozygous allele 1 (1,1) was 29% and that of homozygous allele 2 (2,2) was 6%. In controls, prevalence of IL-1α heterozygous alleles (1,2) was 35%, but no controls had homozygous allele 2 (2,2) (Table 2).

OR for IL-1α allele 2 in the LAgP group was 2.928 (95% CI (1.27 - 6.7), $P = 0.01$).

While the prevalence of IL-1β homozygous allele 1 (1,1) was higher in the LAgP group (62%), the prevalence of IL-1β homozygous allele 2 (2,2) was higher in controls (77%). This difference was statistically significant ($\chi^2 = 41.049, P < 0.001$). In the LAgP group, prevalence of IL-1β heterozygous alleles (1,2) and homozygous allele 2 (2,2) was 35% and 3%, respectively. While the prevalence of the IL-1β heterozygous (1,2) was 23% in control individuals, no controls had homozygous allele 1 (1,1) (Table 3). OR for IL-1β allele 1 in the LAgP group was 29.615 (95% CI (10.936-80.202), $P < 0.0001$).

The prevalence of TNF-α homozygous allele 1 (1,1) was higher in the control group than in the LAgP group (77%; vs 55% $\chi^2 = 4.511, P = 0.105$; not significant; Table 4).

| Table 2 Frequency of IL-1α+$^{4845}$ genotype and alleles in LAgP and controls |
|--------------------------------|----------------|
| **IL-1α** | **Group** | **LAGP (%)** | **Control (%)** |
| n | % | n | % |
| 1/1 | 9* | 29 | 20* | 64.5 |
| 2/2 | 2* | 6 | 0* | 0 |
| 1/2 | 20* | 65 | 11* | 35.5 |
| Total | 31 | 100 | 31 | 100 |
| Allele 1 | 38 | 61.29 | 51 | 82.25 |
| Allele 2 | 24 | 38.7 | 11 | 17.74 |

* $\chi^2 = 8.785, P < 0.05$

| Table 3 Frequency of IL-1β+$^{3954}$ genotype and alleles in LAgP and controls |
|--------------------------------|----------------|
| **IL-1β** | **Group** | **LAGP (%)** | **Control (%)** |
| n | % | n | % |
| 1/1 | 19* | 61.3 | 0* | 0 |
| 2/2 | 1* | 3.2 | 24* | 77.4 |
| 1/2 | 11* | 35.5 | 7* | 22.6 |
| Total | 31 | 100 | 31 | 100 |
| Allele 1 | 49 | 79.03 | 7 | 11.29 |
| Allele 2 | 13 | 20.96 | 55 | 88.7 |

* $\chi^2 = 41.049, P < 0.001$

| Table 4 Frequency of TNF-α+$^{308}$ genotype (not significant) and alleles in LAgP and controls |
|--------------------------------|----------------|
| **TNF-α** | **Group** | **LAGP (%)** | **Control (%)** |
| n | % | n | % |
| 1/1 | 7 | 4.8 | 24 | 77.4 |
| 2/2 | 2 | 6.5 | 0 | 0 |
| 1/2 | 2 | 8.7 | 7 | 22.6 |
| Total | 31 | 100 | 31 | 100 |
| Allele 1 | 46 | 74.19 | 55 | 88.7 |
| Allele 2 | 16 | 28.8 | 7 | 11.29 |

| Table 5 Frequency of TNF-β+$^{3954}$ genotype (not significant) and alleles in LAgP and controls |
|--------------------------------|----------------|
| **TNF-β** | **Group** | **LAGP (%)** | **Control (%)** |
| n | % | n | % |
| 1/1 | 7 | 4.8 | 24 | 77.4 |
| 2/2 | 2 | 6.5 | 0 | 0 |
| 1/2 | 2 | 8.7 | 7 | 22.6 |
| Total | 31 | 100 | 31 | 100 |
| Allele 1 | 46 | 74.19 | 55 | 88.7 |
| Allele 2 | 16 | 28.8 | 7 | 11.29 |
Discussion

Periodontal diseases affect millions of people around the world. Individual factors play an important role in progression of periodontal disease, and host defense in particular is thought to be under genetic control. Studies of AgP (formerly known as juvenile periodontitis) (27) have revealed a genetic basis (1,28,29); however, the specific nature of the genetic risk remains unclear.

Particular cytokine genotypes are thought to directly influence the pathogenesis of inflammatory disease (25). This association is well summarized by numerous authors who describe IL-1 and TNF-α a having a key role in the pathogenesis of periodontal diseases (5,15).

In the present study, we evaluated 31 well-diagnosed Turkish LAgP patients (Fig. 1 shows CAL for the LAgP patients). One study found a prevalence of LAgP of 0.6% in a population of 3056 Turkish adolescents and young adults whose ages ranged between 13 and 19 (30). The LAgP group in the present study represents 5167 randomly selected young adults. According to Kinane and Hart, the numbers of cases and controls in some studies were too small to find an association between IL-1 polymorphisms and periodontal disease (31). Although the small size of such studies means that results are not statistically significant, the results are mathematically significant (32). Multicenter studies are mandatory to obtain an adequate number of patients for accurate comparison of results.

In the literature, LAgP was generally found to be more common in females than males (33-43); indeed, in an unpublished study performed by Saribay et al. (30) in a Turkish population, the female: male ratio was 1.25. However, other studies have noted different gender distributions of LAgP (41,44,45). The findings of our study supported a predilection of LAgP for women; 29 of 31 patients were female. This might be explained by the fact that females tend to be more sensitive about their appearance and more likely to seek dental attention than males. Another possible reason is the earlier age of puberty in females; hormonal changes during the menstrual cycle and pregnancy might aggravate the clinical course of the disease.

Studies of LAgP are difficult to compare due to different diagnostic criteria for LAgP patients, low prevalence of LAgP, unknown periodontal status of control individuals, and varying methodology. The literature contains a limited number of studies investigating genetic polymorphisms in LAgP patients. Since genetic polymorphisms vary in different populations, results do not appear to be applicable across ethnic populations or internationally (18,20,21). Although the Human Genome Project stated the basic genetic similarity of all humans in 2001 (46), subsequent analysis has demonstrated that genetic data can be used to accurately classify humans into populations (47). Studies of population genetics have revealed great genetic variation within racial or ethnic subpopulations, but also substantial variation among the five major racial groups: Caucasians, Africans, Oceanians, East Asians, and Native Americans (48,49). The Turkish ethnicity belongs to the Caucasian racial group, which is the most frequently studied. There are estimated to be at least 15 million genetic polymorphisms, and an as yet undefined subgroup of these polymorphisms underlies variation in normal and disease traits. The importance of such variation is underscored by the fact that a change of only a single base pair can cause many well-known inherited diseases or increase the risk of common disorders.

The literature contains no other data regarding the association of IL-1 and TNF-α genetic polymorphisms with LAgP in a Turkish population. It is necessary to know the prevalence of these polymorphisms in the population to determine the association. For IL-1β+3953, frequencies of allele 1 homozygosity, allele 2 homozygosity, and heterozygosity were 38.7%, 19.6% and 41.7%, respectively in 163 healthy Turkish individuals (50). However, exclusion criteria for control individuals were only Behcet’s Disease history and symptoms of Behcet’s Disease. In a study of 89 healthy Turkish individuals, frequencies of allele 1 homozygosity, allele 2 homozygosity, and heterozygosity for TNF-α-308 were 90%, 0%, and 10%, respectively (51). The main purpose of that study was to evaluate rheumatic fever, so exclusion criteria were only rheumatic fever history and cardiac disease. In another study, frequency of TNF-α-308 allele 1 homozygosity, allele 2 homozygosity, and heterozygosity were 82%, 0.9%, and 17.1% respectively in 108 healthy Turkish individuals (52). The aim of that study was to evaluate cytokine gene polymorphisms in inflammatory bowel disease and controls were described as unrelated healthy volunteers by the authors.

In these studies, the control individuals were considered “otherwise healthy subjects”. Nevertheless, neither medical nor periodontal/dental examinations were performed, so we are unaware of their status in these health fields. In the present study, the control group consisted of dental faculty 5th grade students, and detailed medical, dental and family histories were elicited.

Walker et al. (19) reported that all African-American LAgP patients they studied showed allele 1 gene polymorphism for IL-1β+3953. Pociot et al. (53) reported allele 2 as a rare polymorphism of the IL-1β+3953 gene and suggested that this allele was responsible for increased IL-1β production in LAgP patients. In the present study,
allele 2 gene polymorphism were detected in at least 1 of 31 LAgP patients, and LAgP patients had a higher prevalence of allele 1 for IL-1β+3953. These results are consistent with previous studies. However, Diehl et al. (18) found an association between GAgP and IL-1β+3953 allele 1 polymorphism, but no association between LAgP and IL-1β+3953 allele 1 polymorphism. In a study of Caucasians, Parkhill et al. (20) found an association between IL-1β+3954 allele 1 and LAgP in smokers but not in non-smokers. Our LAgP and control groups both consisted of non-smokers as we aimed to eliminate other predisposing factors for LAgP.

Gonzales et al. (32) compared IL-1α+4845 and IL-1β+3954 genotypes in two populations (European Caucasians and Central American Hispanics) with AgP. For both genotypes, allele 1 was the most common allele, but these results were not statistically significant. While this is consistent with our findings in terms of IL-1β+3954 gene polymorphism, it does not match our findings for IL-1α+4845 gene polymorphism. Quappe et al. (54) found a positive association between AgP and the presence of the IL-1β+3954 allele 2 polymorphism in Chilean patients. However, Hodge et al. (21) and Tai et al. (55) reported no association between IL-1 genotype and GAgP for European Caucasians and Japanese, respectively.

TNF-α has an important role in the pathogenesis of periodontal diseases. TNF-α is a potent immunologic mediator in the proinflammatory process (11). Craandijk et al. (26) reported that the TNF-α gene containing the TNF-α-308 allele has high transcription activity and can accordingly produce a large amount of TNF-α in adults.

Endo et al. (24) and Kinane et al. (23) found no association between GEOP (Generalized Early Onset Periodontitis) and TNF-α gene polymorphism, and Shapira et al. (25) also found no association with EOP (Early Onset Periodontitis). Similarly, the present study found no association between LAgP and TNF-α-308 gene polymorphism.

Proinflammatory cytokines play an essential role in various systemic diseases. Although the IL-1 system is more reliable than other cytokines in revealing strong links for disease pathogenesis, it is not specific for individual body systems and indicates the inflammatory phenotype of the whole body (56). Shapira et al. (57) reported that since neutrophil defects in LJP (Localized Juvenile Periodontitis) patients are intrinsic defects, TNF-α and IL-1β in the serum of LJP patients had a minimal effect on neutrophil function. In the present study, we investigated cytokine gene polymorphisms in peripheral blood. We suggest that specimens obtained from inflammation sites can give us more detailed information about influence of genetic polymorphisms on the inflammatory process. Only one study in the literature investigated particular periodontal tissue parameters associated with periodontal disease (58). That study evaluated genes encoding endogenous mediators of inflammation and structural factors of periodontal tissue. The authors suggested that single nucleotide polymorphisms are the indicators rather the cause of periodontal disease.

The present study, together with the findings published in the literature, shows that polymorphic changes detected in LAgP patients can remain as a risk factor with early and accurate diagnosis, proper treatment, maintenance of oral hygiene and frequent control visits. Polymorphisms are a useful marker for populations; however functional significance of these gene polymorphisms has not yet been confirmed. Prognostic determination is not clear, and longitudinal studies are needed. The importance of genetic polymorphisms in periodontal diseases is based on them being considered risk factors rather than diagnostic criteria. However, identification of the genes and chromosomes responsible for periodontal diseases could allow us to treat affected patients with gene therapy in the future.

Our study provides evidence that the IL-1α and IL-1β gene polymorphisms are associated with LAgP in the Turkish population, in the absence of the other risk factors tested. IL-1 genotype appears to be an important risk factor for LAgP in the Turkish population.

**Acknowledgments**

This study was supported by grant BAP 20030802052 from Ankara University, Ankara, Turkey. The authors would like to thank Zubeyde Arat, MD from Baskent University for statistical analysis and Ms. Mine Guldali for editing the manuscript.

**References**


In the manuscript, the disease has been referred to by different names and abbreviations due to the different classification systems and improved knowledge of the disease pathogenesis during the last era. The authors would like to recommend reference 27 (Guzeldemir and Uslu Toygar, 2006) for further reading.
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