

PST® Update:

Does the addition of IL1RN (“Extended PST” or “PST Plus”) add clinically valuable information to the PST® Test?

Extensive research throughout the world since the early 1990’s has clearly shown that inherited variations in the interleukin-1 (IL-1) genes lead to over- or under-expression of IL-1 and other inflammatory mediators. These same variations have been associated with earlier and/or more severe presentation of several chronic diseases of aging, including chronic adult periodontitis.

Although there are many genes involved in IL-1 biologic activity, the levels of IL-1 activity in most tissues are primarily determined by the genes for IL-1 alpha (IL-1 α) and IL-1 beta (IL-1 β), two similar chemicals that activate inflammation, and the naturally occurring antagonist of inflammation, IL-1 receptor antagonist (IL-1Ra).

Since the first report on the role of IL-1 gene variations in periodontal disease in 1997¹, there have been over 30 papers published on the relationship of these gene variations on various aspects of periodontal disease. Although, some of the papers do not agree with the initial report, the conclusions below are well-supported by multiple studies.

In 1997, Interleukin Genetics analyzed multiple IL-1 gene variations and found one pattern that was most predictive of risk for severe periodontitis in Caucasian adults. That pattern included one marker in the gene for IL-1 α (IL1A+4845; or IL1A-889; they both identify the same genetic pattern) and one marker in the gene for IL-1 β . This combination is trademarked as the PST® test. Other variations in those genes and in the gene for IL-1 receptor antagonist were evaluated but were not found to add value in predicting risk for more severe periodontitis.

PST® positive patients:

- Over produce IL-1, a primary chemical in periodontal bone loss²⁻⁴
- Have higher levels of bacterial pathogens^{5; 6}
- Are more likely to develop severe periodontal disease¹ (confirmed in more than ten studies)
- Are more likely to lose teeth due to periodontal disease⁷⁻¹⁰
- Are more likely to have implant complications¹¹⁻¹³

Laine and co-workers reported in 2001 that a marker in the gene for IL-1Ra (IL1RN+2018, allele 2), when added to the PST markers, was marginally

significant as a predictor of severe periodontitis¹⁴. When the IL-1 genetics were combined with certain bacterial patterns, the “extended” PST that included the IL1RN marker appeared to improve the association with severe periodontitis. Based on these findings, the “PST Plus” test and interpretation were devised, as shown in Table 1.

Collaborators of Interleukin Genetics at the University of Sheffield UK had speculated that susceptibility to inflammatory diseases, such as periodontitis, may be increased in individuals who carry gene polymorphisms that lead to over-expression of IL-1 α and IL-1 β , or who carry gene polymorphisms that lead to under-expression of IL-1Ra gene¹⁵⁻¹⁷. IL1+2018 allele 2 (or the concurrent IL1RN variable number tandem repeat allele 2) gene variation has been associated with lower salivary IL-1Ra and higher serum IL-1Ra in one study¹⁸, but others have reported this genotype to be associated with both increased and decreased levels of IL-1Ra in various tissues¹⁹⁻²².

The “PST Plus” test and interpretation are shown in Table 1.

Table 1

“PST Plus” Risk Type ^a	PST status	IL1RN status	“PST Plus” Interpretation	Frequency in Caucasians ^b
Type 1	Negative	Negative	Negative	0.323
Type 2	Positive	Negative	Positive	0.208
Type 3	Positive	Positive	Positive	0.143
Type 4	Negative	Positive	Positive	0.326

^a www.Hain-lifescience.de

^b Interleukin Genetics, unpublished data from 900 Caucasians of unspecified periodontal status

The “PST Plus” risk types 2 & 3 above (Table 1) would be “positive” for both PST® and PST Plus. Risk type 1 would be negative for both PST and PST Plus. The only practical clinical difference between PST® and PST Plus is the type 4 group above, in which the patient would receive a PST® negative test results, but would receive a positive test result on the PST Plus test. This means that, using the PST Plus test, the patient would be classified as increased risk for severe periodontitis based on IL1RN+2018 allele 2 alone. **The addition of type 4 to the genetic risk assessment test does not appear to be justified for the following reasons:**

1. the addition of the type 4 category would classify close to 70% of the Caucasian population (Types 2+3+4 = 67.7%) as being high risk for severe periodontitis. This is in total contradiction to both epidemiological studies and the clinical experience of most dentists for the past 30 years. Most data and experience indicate that approximately 30% of the adult population has any type of periodontitis, with approximately 15%

developing moderate to severe destruction.

2. the data supporting increased risk due to IL1RN+2018 allele 2 alone (risk type 4) are limited and contradictory (see table 2)

Table 2

Reference	Population tested	PST AND IL1RN+2018 allele 2 ^a —Increased risk ^b	IL1RN+2018 allele 2 ^a alone—Increased risk ^b
Kornman 1997 ¹	Caucasians U.S.; adult periodontitis	No	No
Laine 2001 ¹⁴	Caucasians Europe; adult periodontitis	Yes	No
Meisel 2002 ²³	Caucasians Europe; adult periodontitis	Yes	No
Tai 2002 ²⁴	Japanese; generalized aggressive periodontitis	No	Yes
Parkhill 2000 ²⁵	Caucasians Europe; aggressive periodontitis	Significant decreased risk	Significant decreased risk
Laine 2006 ²⁶	Caucasians Europe; peri-implantitis	Yes	Yes
Berdeli 2006 ²⁷	Caucasians Turkey; adult & generalized aggressive periodontitis	Not tested	Yes
Dashash 2007 ²⁸	Caucasians Europe; gingivitis in children	Not tested	Significant decreased risk

^a Either IL1RN+2018 allele 2 or IL1RN variable number tandem repeat (VNTR) allele 2

^b Increased risk may be limited to smokers or non-smokers

Summary: Relevant available data predict that the main effect of adding IL1RN to the PST® test is to increase false positives---i.e. it would increase the number of patients classified as high risk even though they are not really at increased risk. Thus, based on currently available data, the “PST Plus” test appears to substantially over-estimate risk---i.e. it labels almost 70% of adults as being at high risk for severe periodontitis. In addition, there is no persuasive evidence that the addition of IL1RN would detect patients at true increased risk who are not already detected by the PST® test.

In general, there is insufficient clinical evidence to justify the addition of the IL1RN+2018 polymorphism to the current PST® test.

References:

1. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW *et al.* The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997; **24**(1): 72-77.
2. Engebretson SP, Lamster IB, Herrera-Abreu M, Celenti RS, Timms JM, Chaudhary AG *et al.* The influence of interleukin gene polymorphism on expression of interleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. *J Periodontol* 1999; **70**(6): 567-573.
3. Shirodaria S, Smith J, McKay IJ, Kennett CN & Hughes FJ. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. *J Dent Res* 2000; **79**(11): 1864-1869.
4. Rogus J, Beck JD, Offenbacher S, Huttner K, Iacoviello L, Latella MC *et al.* IL1B gene promoter haplotype pairs predict clinical levels of interleukin-1beta and C-reactive protein. *Hum Genet* 2008; **123**(4): 387-398.
5. Socransky SS, Haffajee AD, Smith C & Duff GW. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. *J Clin Periodontol* 2000; **27**(11): 810-818.
6. Kowalski J, Gorska R, Dragan M & Kozak I. Clinical state of the patients with periodontitis, IL-1 polymorphism and pathogens in periodontal pocket- is there a link? (An introductory report). *Adv Med Sci* 2006; **51 Suppl 1**: 9-12.
7. Eickholz P, Kaltschmitt J, Berbig J, Reitmeir P & Pretzl B. Tooth loss after active periodontal therapy. 1: patient-related factors for risk, prognosis, and quality of outcome. *J Clin Periodontol* 2008; **35**(2): 165-174.
8. McGuire MK & Nunn ME. Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. *J Periodontol* 1999; **70**(1): 49-56.
9. Axelsson P. Role of genetic and hereditary factors *Diagnosis and risk prediction of periodontal diseases*, vol. 3. Quintessence: Carol Stream, 2002, pp 146-163.

10. Struch F, Dau M, Schwahn C, Biffar R, Kocher T & Meisel P. Interleukin-1 gene polymorphism, diabetes, and periodontitis: results from the Study of Health in Pomerania (SHIP). *J Periodontol* 2008; **79**(3): 501-507.
11. Feloutzis A, Lang NP, Tonetti MS, Burgin W, Bragger U, Buser D *et al.* IL-1 gene polymorphism and smoking as risk factors for peri-implant bone loss in a well-maintained population. *Clin Oral Implants Res* 2003; **14**(1): 10-17.
12. Gruica B, Wang HY, Lang NP & Buser D. Impact of IL-1 genotype and smoking status on the prognosis of osseointegrated implants. *Clin Oral Implants Res* 2004; **15**(4): 393-400.
13. Jansson H, Hamberg K, De Bruyn H & Bratthall G. Clinical consequences of IL-1 genotype on early implant failures in patients under periodontal maintenance. *Clin Implant Dent Relat Res* 2005; **7**(1): 51-59.
14. Laine ML, Farre MA, Gonzalez G, van Dijk LJ, Ham AJ, Winkel EG *et al.* Polymorphisms of the interleukin-1 gene family, oral microbial pathogens, and smoking in adult periodontitis. *J Dent Res* 2001; **80**(8): 1695-1699.
15. Duff GW. Genetic variation in cytokines and relevance to inflammation and disease. In: Balkwill F (ed). *The cytokine network. Frontiers in molecular biology*, vol. 25. Oxford University Press: Oxford, 2000, p 152.
16. Duff GW. Influence of genetics on disease susceptibility and progression. *Nutr Rev* 2007; **65**(12 Pt 2): S177-181.
17. Kornman KS & di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Ann Periodontol* 1998; **3**(1): 327-338.
18. Perrier S, Coussediere C, Dubost JJ, Albuissou E & Sauvezie B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjogren's syndrome and rheumatoid arthritis. *Clin Immunol Immunopathol* 1998; **87**(3): 309-313.
19. Reiner AP, Wurfel MM, Lange LA, Carlson CS, Nord AS, Carty CL *et al.* Polymorphisms of the IL1-receptor antagonist gene (IL1RN) are associated with multiple markers of systemic inflammation. *Arterioscler Thromb Vasc Biol* 2008; **28**(7): 1407-1412.
20. Rafiq S, Stevens K, Hurst AJ, Murray A, Henley W, Weedon MN *et al.* Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. *Genes Immun* 2007; **8**(4): 344-351.

21. Hurme M & Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. *Eur J Immunol* 1998; **28**(8): 2598-2602.
22. Danis VA, Millington M, Hyland VJ & Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. *Clin Exp Immunol* 1995; **99**(2): 303-310.
23. Meisel P, Siegemund A, Dombrowa S, Sawaf H, Fanghaenel J & Kocher T. Smoking and polymorphisms of the interleukin-1 gene cluster (IL-1alpha, IL-1beta, and IL-1RN) in patients with periodontal disease. *J Periodontol* 2002; **73**(1): 27-32.
24. Tai H, Endo M, Shimada Y, Gou E, Orima K, Kobayashi T *et al.* Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *J Clin Periodontol* 2002; **29**(10): 882-888.
25. Parkhill JM, Hennig BJ, Chapple IL, Heasman PA & Taylor JJ. Association of interleukin-1 gene polymorphisms with early-onset periodontitis. *J Clin Periodontol* 2000; **27**(9): 682-689.
26. Laine ML, Leonhardt A, Roos-Jansaker AM, Pena AS, van Winkelhoff AJ, Winkel EG *et al.* IL-1RN gene polymorphism is associated with peri-implantitis. *Clin Oral Implants Res* 2006; **17**(4): 380-385.
27. Berdeli A, Emingil G, Gurkan A, Atilla G & Kose T. Association of the IL-1RN2 allele with periodontal diseases. *Clin Biochem* 2006; **39**(4): 357-362.
28. Dashash M, Drucker DB, Hutchinson IV, Bazrafshani MR & Blinkhorn AS. Interleukin-1 receptor antagonist gene polymorphism and gingivitis in children. *Oral Dis* 2007; **13**(3): 308-313.