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## CLINICAL AND PSYCHOEMOTIONAL PHENOTYPIC SIGNATURE OF THE COMBINED RECESSIVE TLR4 RS1927911 AND RS2149356 GENOTYPIC PROFILE IN PATIENTS WITH PERIODONTITIS

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The study characterized the clinical phenotype associated with the combined recessive TLR4 profile (A/A rs1927911 and/or T/T rs2149356). A prespecified secondary analysis included 184 patients with periodontitis, including 16 profile carriers. Structural, inflammatory, oral hygiene, psychoemotional, behavioral, and anamnestic characteristics were assessed using block-wise FDR adjustment and regression models. Carriers more frequently had generalized disease, stages II–III, and morphometrically defined grades B–C, greater periodontal destruction, higher inflammatory and plaque indices, and more pronounced anxiety and posttraumatic symptoms. Among ever-smokers, cumulative tobacco exposure was greater in carriers. The proposed integrative inflammatory-hygiene index was higher in carriers and associated with unfavorable clinical categories. The findings identify a complex phenotypic signature but remain hypothesis-generating because of the small, exclusively male carrier group and cross-sectional design. Independent replication and external validation of the index are required.

**Key words:** periodontitis, diagnosis, TLR4, single nucleotide polymorphism, genotype–phenotype associations, phenotypic signature, psychoemotional status, personalized dentistry, treatment of periodontal diseases.

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## КЛІНІКО-ПСИХОЕМОЦІЙНА ФЕНОТИПОВА СИГНАТУРА ОБ'ЄДНАНОГО РЕЦЕСИВНОГО ГЕНОТИПОВОГО ПРОФІЛЮ TLR4 RS1927911 І RS2149356 У ПАЦІЄНТІВ ІЗ ПАРОДОНТИТОМ

Дослідження присвячене характеристиці клінічного фенотипу, асоційованого з об'єднаним рецесивним профілем TLR4 (A/A rs1927911 та/або T/T rs2149356). Вторинний попередньо спланований аналіз охопив 184 пацієнтів із пародонтитом, зокрема 16 носіїв цього профілю. Оцінювали структурні, запальні, гігієнічні, психоемоційні, поведінкові й анамнестичні характеристики із застосуванням блокової FDR-корекції та регресійних моделей. У носіїв частіше виявляли генералізований процес, стадії II–III, ступені B–C за морфометричним критерієм, виразнішу деструкцію пародонту, вищі запальні й гігієнічні індекси, тривогу та посттравматичні симптоми. Серед осіб, які курили будь-коли, носії мали вищу накопичену тютюнову експозицію. Запропонований інтегративний запально-гігієнічний індекс був вищим у носіїв і асоціювався з несприятливими клінічними категоріями. Виявлено комплексну фенотипову сигнатуру, проте результати мають гіпотезотвірне значення через малу, виключно чоловічу групу носіїв і поперечний дизайн. Необхідні незалежне відтворення результатів та зовнішня валідація індексу.

**Ключові слова:** пародонтит, діагностика, TLR4, одонуклеотидний поліморфізм, генотип-фенотипові асоціації, фенотипова сигнатура, психоемоційний стан, персоналізована стоматологія, лікування захворювань пародонту.

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Periodontitis is a multifactorial inflammatory disease whose clinical course varies substantially among patients. According to the consensus report by P.N. Papapanou et al. [17], the diagnosis of periodontitis is based on clinical attachment loss that cannot be attributed to other causes, while M.S. Tonetti et al. [19] proposed distinguishing disease extent, stage, and grade. These characteristics describe different aspects of established disease but do not explain the full variability in inflammation, oral hygiene, and subjective manifestations.

Microbial biofilm remains an essential component of pathogenesis, but its effects depend on the host response. G. Hajishengallis and T. Chavakis [10] showed that disrupted immune homeostasis sustains local inflammation and may link periodontitis to systemic inflammatory conditions. Differences in responses to similar microbial loads may partly reflect genetic variability in innate immune receptors.

Contemporary genomic studies support the polygenic nature of periodontitis, although the reproducibility of individual findings remains limited. A systematic review by C. Gao et al. [9] demonstrated substantial heterogeneity in phenotypes, populations, and statistical approaches across genomic studies. H. Dommisch et al. [7] likewise concluded that no single genetic marker currently has sufficient evidence to support its clinical use. This highlights the need for more rigorous evaluation of genotype–disease associations and more differentiated characterization of carrier phenotypes.

In this context, the Toll-like receptor 4 (TLR4) gene is a biologically plausible candidate. TLR4 is a key component of the innate immune system responsible for recognizing bacterial endotoxins, particularly lipopolysaccharides, and initiating a

cascade of systemic inflammatory responses [4, 11]. Direct periodontal evidence concerning rs1927911 and rs2149356 remains sparse. W. Li et al. [12] described recessive associations in a subgroup of *Porphyromonas gingivalis*-positive patients. A. Abou-Bakr et al. [1] investigated rs2149356 in patients with periodontitis and end-stage renal disease, although this specific clinical context limits the direct generalizability of their findings.

Psychoemotional status represents a distinct dimension of the phenotype. J. Ball and I. Darby [3] summarized evidence linking mental disorders and stress to more severe periodontal disease through behavioral and immune-inflammatory mechanisms. In a cross-sectional study by C. Marruganti et al. [13], the combination of stress and poor sleep quality was associated with more severe periodontitis. According to a systematic review by E.K. Mauland and S.P. Neupane [15], psychoemotional distress may also compromise periodontal treatment outcomes. However, the relationships among TLR4 genotype, inflammatory and oral hygiene parameters, and posttraumatic symptoms remain largely unexplored.

**The purpose** of the study was to characterize the clinical phenotype associated with the combined recessive TLR4 profile (A/A rs1927911 and/or T/T rs2149356) in patients with periodontitis and to develop an exploratory genotype–phenotype model.

**Materials and methods.** The study complied with the Declaration of Helsinki and Good Clinical Practice and was approved by the Bioethics Committee of the Private Higher Education Institution ‘Kyiv Medical University’ (March 7, 2024; Protocol No. 21); all patients gave informed consent to participation and data use. This prespecified secondary analysis used a single-center, cross-sectional clinical-genetic cohort of patients treated at the university dental clinic (Kyiv, Ukraine) under martial law between April 1, 2024 and April 1, 2026.

Inclusion criteria were age  $\geq 18$  years, birth and permanent residence in Ukraine, presence in the country during active hostilities since February 2022, and a completed comprehensive periodontal examination; exclusion criteria were severe decompensated systemic disease, cancer, lack of informed consent, or incomplete medical records. Periodontitis was classified according to the 2017 World Workshop system [17, 19] as localized ( $<30\%$  of teeth) or generalized ( $\geq 30\%$ ) and staged by severity and management complexity. Without longitudinal data, grade was determined by the ratio of radiographic bone loss to age (RBL/age) [19]. The grade modifiers smoking and diabetes mellitus [5] were not applied, to avoid definitional circularity when analyzing tobacco exposure as a phenotypic characteristic, and diabetes was too rare for valid analysis.

Of the 196 patients with confirmed periodontitis (164 [83.7%] men), 184 had analyzable genotype data (12 excluded for incomplete or technically

inconsistent genotyping): 152 (82.6%) men and 32 (17.4%) women, mean age  $38.8 \pm 9.6$  years; 136 (73.9%) were current or former smokers, and one had diabetes mellitus. Localized/generalized disease was present in 100 (54.3%)/84 (45.7%), stage I/stages II–III in 70 (38.0%)/114 (62.0%), and grade A/grades B–C in 25 (13.6%)/159 (86.4%) patients.

The definitional structural block comprised clinical attachment loss (CAL), probing pocket depth (PPD), radiographic bone loss (RBL), RBL/age, furcation involvement, and tooth mobility, which define stage or grade and were used to assess internal consistency. The primary non-definitional block included exudation, bleeding on probing (BoP), the papillary-marginal-alveolar index (PMA), the Schiller–Pisarev test, and the OHI-S (Green–Vermillion), O’Leary, and Turesky oral hygiene indices. Psychoemotional status was assessed with the Hamilton Anxiety Rating Scale and the Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5;  $\geq 33$  points indicated probable posttraumatic stress disorder). Demographic-behavioral variables (age, sex, ever-smoking, pack-years, alcohol, coffee, sleep disturbances, dental care before martial law, and combat injury), nine comorbidity categories from medical history, and nine complaints were analyzed separately.

Genomic DNA was isolated from 3 mL of whole venous blood (PureLink® Genomic DNA Kit, Invitrogen, USA), and TLR4 rs1927911 (G/A; A8595G) and rs2149356 (G/T) were genotyped by real-time polymerase chain reaction with TaqMan Mutation Detection Assays (Life Technologies, USA) on a Gene Amp® PCR System 7500 (Applied Biosystems, USA). Genotyping succeeded for rs1927911 in 182, rs2149356 in 190, and both loci in 178 of the 196 patients. Group 1 (combined recessive profile) comprised 16 carriers of A/A rs1927911 and/or T/T rs2149356 (10 double minor homozygotes and three A/A and three T/T carriers with the other locus undetermined); Group 2 comprised 168 patients without either minor homozygous genotype.

Statistical analysis was performed in R 4.3.3. Data are presented as mean  $\pm$  standard deviation (age), median [Q1–Q3], or  $n$  (%). Groups were compared with the two-sided Mann–Whitney test with correction for ties and Fisher’s exact test; effect size was expressed as Cliff’s  $\delta$  (the difference in proportions for binary variables) with 95% confidence intervals (CIs) by Newcombe’s method or stratified nonparametric bootstrap (10,000 resamples). Multiplicity was controlled by the Benjamini–Hochberg method within six prespecified blocks (definitional structural, primary non-definitional, psychoemotional, demographic-behavioral, somatic, and complaints), with  $q < 0.05$  considered robust within a block; blocks other than the primary non-definitional and structural blocks were exploratory.

The prespecified integrative inflammatory-hygiene index (IIHI) combined an inflammatory

domain (BoP, exudation, PMA, and the Schiller–Pisarev test) and an oral hygiene domain (OHI-S, O’Leary, and Turesky): each measure was converted to a within-sample percentile and averaged within its domain, and the two standardized domain scores were combined with equal weights and restandardized, so that one unit corresponded to a one-standard-deviation deterioration. The IIHI is a research measure without an established clinical threshold.

The association between genotype group and the IIHI was assessed by linear regression adjusted for age, sex, and smoking, with HC3 heteroscedasticity-consistent standard errors to account for unequal residual variances and group sizes. The three clinical axes were analyzed by Firth’s bias-reduced logistic regression adjusted for age, sex, and smoking; age was omitted from the primary grade models because grade was defined through RBL/age (an age-adjusted model was a sensitivity analysis).

The inflammatory domain, oral hygiene domain, or IIHI was added sequentially, and changes in the profile log(OR) were evaluated descriptively, not as formal mediation. Sensitivity analyses replaced smoking with log(1 + pack-years) or were restricted to the 178 patients with complete genotype pairs. STREGA recommendations were considered in reporting.

**Results of the study.** The combined recessive group was defined because minor homozygotes at both loci showed nominal same-direction associations with generalized disease in a preliminary analysis of this cohort. In each minor homozygous group (A/A rs1927911 and T/T rs2149356; Table 1), generalized disease was present in 76.9 % (10/13), stages II–III in 84.6 % (11/13), and grades B–C in 100.0 % (13/13), and median CAL, PPD, BoP, PMA, OHI-S, O’Leary, anxiety, and PCL-5 values exceeded those of the other genotypes.

Table 1

**Clinical axes and key phenotypic measures by rs1927911 and rs2149356 genotype**

| Parameter                                        | Major homozygote    | Heterozygote        | Minor homozygote    |
|--------------------------------------------------|---------------------|---------------------|---------------------|
| rs1927911: G/G (n=107) / A/G (n=62) / A/A (n=13) |                     |                     |                     |
| Age, years                                       | 39.0 [30.0–45.0]    | 40.5 [31.2–46.8]    | 47.0 [39.0–50.0]    |
| Generalized disease                              | 46/107 (43.0 %)     | 27/62 (43.5 %)      | 10/13 (76.9 %)      |
| Stages II–III                                    | 63/107 (58.9 %)     | 39/62 (62.9 %)      | 11/13 (84.6 %)      |
| Grades B–C                                       | 89/107 (83.2 %)     | 55/62 (88.7 %)      | 13/13 (100.0 %)     |
| CAL, mm                                          | 2.52 [1.99–3.00]    | 2.60 [1.92–3.20]    | 3.50 [2.50–5.20]    |
| PPD, mm                                          | 3.44 [3.02–3.80]    | 3.50 [3.20–3.77]    | 4.50 [3.92–5.50]    |
| RBL, %                                           | 15.2 [9.30–18.8]    | 15.3 [11.5–18.1]    | 18.8 [15.7–33.2]    |
| RBL/age                                          | 0.381 [0.312–0.487] | 0.388 [0.311–0.453] | 0.511 [0.375–0.650] |
| BoP, %                                           | 24.7 [20.8–30.1]    | 24.9 [21.7–30.0]    | 36.9 [23.8–51.2]    |
| PMA, %                                           | 20.1 [15.9–25.3]    | 20.9 [16.4–24.4]    | 26.4 [20.7–38.2]    |
| OHI-S index, points                              | 2.00 [1.90–2.20]    | 2.00 [1.82–2.20]    | 2.40 [2.00–2.80]    |
| O’Leary index, %                                 | 41.0 [35.0–47.5]    | 41.0 [32.2–48.0]    | 48.0 [42.0–54.0]    |
| Turesky index                                    | 1.90 [1.67–2.10]    | 1.90 [1.70–2.10]    | 1.90 [1.80–2.50]    |
| Hamilton Anxiety Rating Scale, points            | 9.00 [5.00–10.0]    | 9.00 [5.00–11.8]    | 11.0 [10.0–22.0]    |
| PCL-5, points                                    | 11.0 [10.0–15.0]    | 12.0 [9.25–16.8]    | 16.0 [13.0–42.0]    |
| rs2149356: G/G (n=96) / G/T (n=81) / T/T (n=13)  |                     |                     |                     |
| Age, years                                       | 38.0 [30.0–45.0]    | 41.0 [33.0–47.0]    | 42.0 [37.0–50.0]    |
| Generalized disease                              | 40/96 (41.7 %)      | 38/81 (46.9 %)      | 10/13 (76.9 %)      |
| Stages II–III                                    | 56/96 (58.3 %)      | 51/81 (63.0 %)      | 11/13 (84.6 %)      |
| Grades B–C                                       | 81/96 (84.4 %)      | 70/81 (86.4 %)      | 13/13 (100.0 %)     |
| CAL, mm                                          | 2.50 [1.99–3.00]    | 2.60 [1.99–3.30]    | 3.50 [2.50–5.20]    |
| PPD, mm                                          | 3.40 [3.00–3.80]    | 3.50 [3.20–3.80]    | 4.96 [3.92–5.50]    |
| RBL, %                                           | 15.2 [9.57–19.2]    | 15.2 [11.2–17.8]    | 18.8 [15.7–33.4]    |
| RBL/age                                          | 0.390 [0.320–0.503] | 0.381 [0.300–0.447] | 0.633 [0.364–0.676] |
| BoP, %                                           | 24.4 [20.6–29.5]    | 25.1 [21.8–30.6]    | 44.2 [24.1–53.2]    |
| PMA, %                                           | 20.0 [15.3–24.8]    | 20.9 [17.6–24.3]    | 33.7 [20.7–41.6]    |
| OHI-S, points                                    | 2.00 [1.90–2.20]    | 2.00 [1.80–2.20]    | 2.40 [2.00–2.80]    |
| O’Leary index, %                                 | 41.5 [34.8–47.2]    | 41.0 [33.0–48.0]    | 51.0 [42.0–54.0]    |
| Turesky index                                    | 1.90 [1.64–2.10]    | 1.90 [1.70–2.13]    | 1.90 [1.80–2.70]    |
| Hamilton Anxiety Rating Scale, points            | 9.00 [5.00–10.0]    | 9.00 [5.00–16.0]    | 11.0 [10.0–22.0]    |
| PCL-5, points                                    | 11.0 [9.75–14.2]    | 13.0 [10.0–28.0]    | 16.0 [13.0–42.0]    |

Notes: Data are presented as median [Q1–Q3] or n/N (%). Formal comparisons among the three genotypes were not performed. II–III: combined stages II and III; B–C: combined grades B and C; CAL: clinical attachment loss; PPD: probing pocket depth; RBL: radiographic bone loss; BoP: bleeding on probing; PMA: papillary-marginal-alveolar index.

In Group 1, generalized disease was present in 75.0 % (12/16) of patients, stages II–III in 81.3 % (13/16), and grades B–C in 100.0 % (16/16), versus 42.9 % (72/168), 60.1 % (101/168), and 85.1 % (143/168) in Group 2. All Group 1 patients were men.

Group 1 showed a consistently unfavorable phenotypic signature (Table 2; Fig. 1): all structural measures were higher or more frequent (all  $q < 0.05$ ; largest effect for PPD,  $\delta = 0.57$ ; 95 % CI 0.28–0.83).

Table 2

## Main components of the phenotypic signature of the combined recessive TLR4 genotype

| Parameter                                 | Group 2             | Group 1             | Cliff's $\delta$ (95 % CI) | p      | q      |
|-------------------------------------------|---------------------|---------------------|----------------------------|--------|--------|
| Definitional structural phenotype         |                     |                     |                            |        |        |
| CAL, mm                                   | 2.59 [1.99–3.10]    | 3.85 [2.50–4.99]    | 0.50 (0.18–0.78)           | 0.001  | 0.002  |
| PPD, mm                                   | 3.50 [3.09–3.80]    | 4.65 [3.77–5.43]    | 0.57 (0.28–0.83)           | <0.001 | <0.001 |
| RBL, %                                    | 15.2 [10.1–18.3]    | 20.6 [15.5–33.2]    | 0.48 (0.20–0.73)           | 0.002  | 0.002  |
| RBL/age                                   | 0.386 [0.310–0.485] | 0.572 [0.372–0.677] | 0.39 (0.10–0.66)           | 0.009  | 0.009  |
| Furcation involvement                     | 2/168 (1.2 %)       | 4/16 (25.0 %)       | 0.24 (0.09–0.48)           | <0.001 | 0.002  |
| Tooth mobility                            | 4/168 (2.4 %)       | 4/16 (25.0 %)       | 0.23 (0.07–0.47)           | 0.002  | 0.003  |
| Primary non-definitional phenotype        |                     |                     |                            |        |        |
| IIHI, SD                                  | –0.19 [–0.91–0.71]  | 0.90 [–0.16–1.72]   | 0.47 (0.19–0.73)           | 0.002  | 0.005  |
| BoP, %                                    | 24.7 [21.3–29.9]    | 40.5 [24.0–52.4]    | 0.50 (0.19–0.77)           | 0.001  | 0.004  |
| PMA, %                                    | 20.2 [15.9–24.8]    | 30.1 [20.7–39.1]    | 0.50 (0.24–0.74)           | <0.001 | 0.004  |
| Schiller–Pisarev test, 1–3                | 1.00 [1.00–2.00]    | 2.00 [1.00–2.00]    | 0.33 (0.07–0.59)           | 0.006  | 0.008  |
| OHI-S, points                             | 2.00 [1.90–2.20]    | 2.45 [2.00–2.80]    | 0.45 (0.15–0.72)           | 0.003  | 0.006  |
| O'Leary index, %                          | 41.0 [34.0–48.0]    | 49.5 [41.5–55.0]    | 0.43 (0.14–0.69)           | 0.004  | 0.007  |
| Psychoemotional phenotype                 |                     |                     |                            |        |        |
| Hamilton Anxiety Rating Scale, points     | 9.00 [5.00–10.3]    | 10.5 [9.8–22.0]     | 0.39 (0.09–0.65)           | 0.010  | 0.015  |
| PCL-5, points                             | 12.0 [9.8–15.0]     | 15.5 [13.0–42.5]    | 0.40 (0.11–0.65)           | 0.008  | 0.015  |
| PCL-5 threshold $\geq 33$                 | 31/168 (18.5 %)     | 6/16 (37.5 %)       | 0.19 (–0.01–0.43)          | 0.097  | 0.097  |
| Other clinically relevant measures        |                     |                     |                            |        |        |
| Pack-years among ever-smokers             | 4.45 [2.80–8.03]    | 13.75 [8.85–22.50]  | 0.64 (0.33–0.86)           | <0.001 | 0.003  |
| Number of recorded symptomatic complaints | 1.00 [1.00–1.00]    | 1.00 [1.00–2.00]    | 0.31 (0.10–0.54)           | 0.002  | 0.018  |

Note: Quantitative and ordinal data are presented as median [Q1–Q3], and binary data as n/N (%). Positive  $\delta$  indicates a higher level or proportion of the characteristic in the profile group. q is the p-value adjusted using the Benjamini–Hochberg method within the corresponding prespecified block. PCL-5  $\geq 33$  is the screening threshold for probable posttraumatic stress disorder; complaints were counted from a prespecified list.

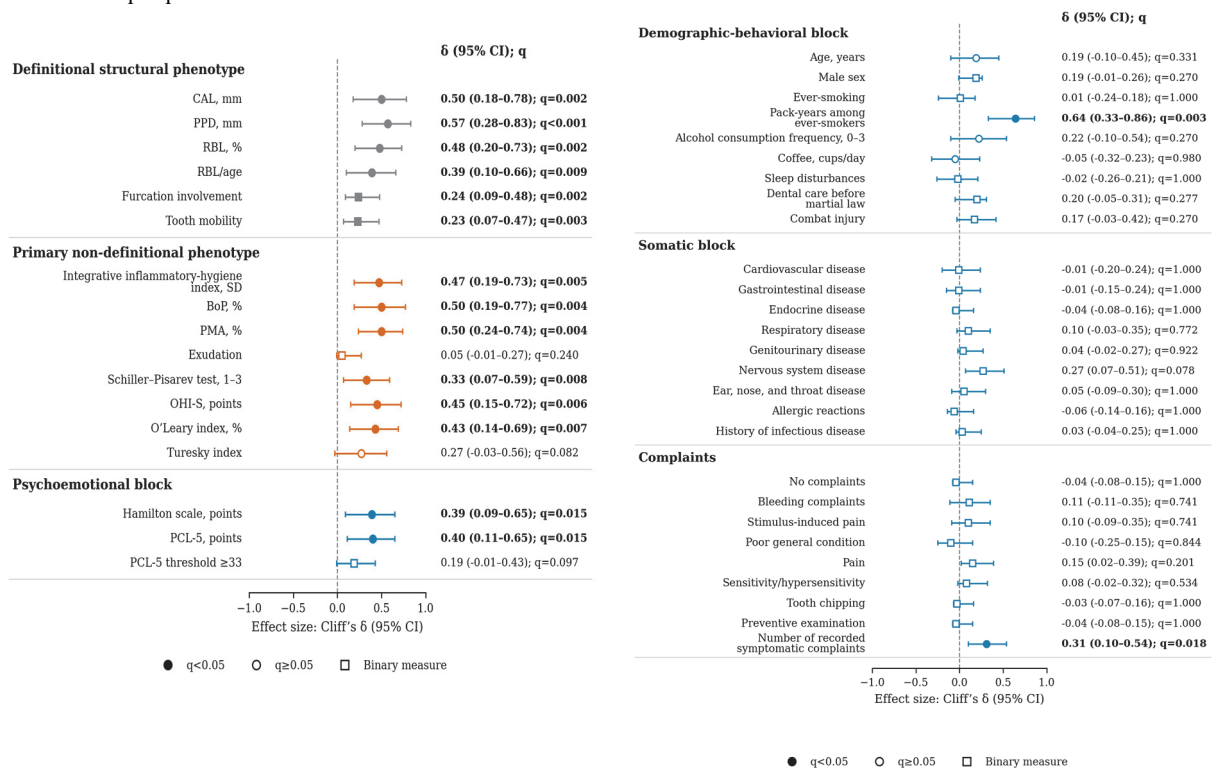


Fig. 1. A – Structural, primary non-definitional, and psychoemotional blocks. B – Phenotypic signature of the combined recessive TLR4 genotype. Cliff's  $\delta$  with 95 % CIs is shown for 44 comparisons. A positive value indicates a higher level of a quantitative or ordinal characteristic, or a greater proportion of a binary characteristic, in Group 1. Filled markers indicate  $q < 0.05$  after block-wise adjustment; open markers indicate  $q \geq 0.05$ .

In the primary non-definitional block, five of seven components showed moderate effects ( $\delta=0.33-0.50$ ;  $q<0.05$ ), whereas exudation and the Turesky index showed smaller, non-robust effects (Fig. 1). The IIHI median was 0.90 standard deviations (SD) in Group 1 versus  $-0.19$  SD in Group 2 ( $\delta=0.47$ ;  $q=0.005$ ); the adjusted difference was  $+0.70$  SD (95 % CI 0.16–1.24;  $p=0.011$ ), or  $+0.63$  SD (95 % CI 0.08–1.17;  $p=0.023$ ) with  $\log(1 + \text{pack-years})$ .

Hamilton Anxiety Rating Scale and PCL-5 scores were also higher in Group 1 (both  $q=0.015$ ; Table 2). The screening threshold for probable posttraumatic stress disorder was met by 37.5 % (6/16) versus 18.5 % (31/168) of patients, a non-significant difference ( $p=0.097$ ).

Current or former smoking was nearly equally frequent, 75.0 % (12/16) versus 73.8 % (124/168), but among ever-smokers pack-years were higher in Group 1: 13.75 [8.85–22.50] versus 4.45 [2.80–8.03] ( $\delta=0.64$ ;  $q=0.003$ ). Group 1 also reported more recorded symptomatic complaints ( $q=0.018$ ).

In the exploratory genotype–phenotype model (Fig. 2), each 1-SD increase in the IIHI was associated with higher odds of generalized disease (OR 2.84; 95 % CI 1.87–4.30), stages II–III (OR 69.41; 95 % CI 17.27–278.99), and grades B–C (OR 7.63; 95 % CI 3.18–18.32; all  $p<0.001$ ) after adjustment for sex, smoking status, genotype group, and, for extent and stage, age. The stage estimate, reflecting strong separation of the categories, is imprecise (wide CI).

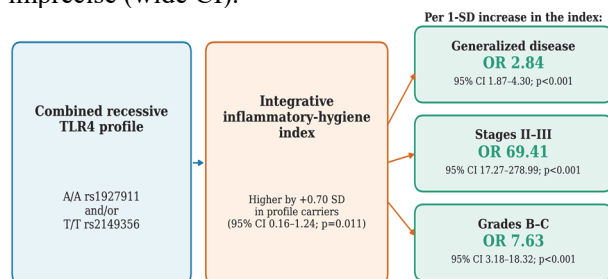


Fig. 2. Exploratory, non-causal framework of the relationships among the combined recessive TLR4 profile, the integrative inflammatory-hygiene index, and the clinical axes of periodontitis. The index difference is adjusted for age, sex, and smoking; odds ratios per 1-SD increase in the index are adjusted for sex, smoking, genotype group, and, for extent and stage, age.

**Discussion.** The key finding was the consistently unfavorable phenotype of minor homozygotes at both loci; the concurrent increase in CAL, RBL, furcation involvement, and tooth mobility indicates structural

destruction rather than inflammatory edema alone. W. Li et al. [12] found recessive signals for both variants in *Porphyromonas gingivalis*-positive individuals, and M.M. Farouk et al. [8] reported a higher frequency of T/T rs2149356 in chronic periodontitis; our data extend these genotype-frequency observations to an integrated phenotype.

This structural component is biologically plausible: lipopolysaccharide-induced TLR4 signaling inhibits the osteogenic differentiation of periodontal ligament stem cells [20], and TLR4 deficiency reduces alveolar bone destruction by shifting the balance between osteoclastogenesis and osteoblastogenesis [16]. Functional data are, however, lacking for rs1927911 and limited for rs2149356, and the altered lipopolysaccharide sensitivity shown for rs4986790 [6] cannot be extrapolated to these intronic variants; the profile may reflect a regulatory effect or linkage with other functional variants.

The non-definitional differences suggest both a stronger inflammatory response and poorer biofilm control. Higher pack-years in carriers may have added to attachment and bone loss; this effect could not be separated from that of genotype, and the small group precluded testing a genotype-by-smoking interaction.

Higher anxiety and PCL-5 scores agree with evidence linking stress-related elevation of salivary cortisol to periodontitis [2], combat-related posttraumatic stress disorder to poorer oral care [18], and periodontal dysbiosis and systemic inflammation to anxiety- and stress-related disorders in both directions [14]; in our cohort, they may also reflect shared war-related stress.

Clinically, the findings identify a genetically defined subgroup with a complex unfavorable phenotype, support assessment beyond stage and grade, and justify further evaluation of TLR4 genotyping as a promising clinical laboratory test. The study's strengths include block-wise multiplicity control and the separation of definitional and non-definitional characteristics.

**Limitations.** The profile group comprised only 16 individuals, all men, limiting precision and the separation of genotype and sex effects despite adjustment for sex; the single-center cross-sectional design precludes causal or mediating inferences, and the IIHI requires external validation.

## Conclusions

1. The minor homozygous genotypes A/A rs1927911 and T/T rs2149356 and the combined recessive TLR4 profile were characterized by a consistently unfavorable phenotype: generalized disease in 75.0 %, stages II–III in 81.3 %, and grades B–C in 100.0 % of profile carriers versus 42.9 %, 60.1 %, and 85.1 % of other patients, with more pronounced structural changes.

2. Carriers had higher BoP, PMA, OHI-S, and O'Leary values, and the proposed integrative inflammatory-hygiene index was 0.70 SD higher after adjustment (95 % CI 0.16–1.24).

3. Anxiety and PCL-5 scores were higher in carriers ( $\delta=0.39$  and  $0.40$ ;  $q=0.015$ ); the screening threshold for probable posttraumatic stress disorder was met by 37.5 % versus 18.5 % of other patients ( $p=0.097$ ).

4. Each 1-SD increase in the index was associated with higher adjusted odds of generalized disease (OR 2.84), stages II–III (OR 69.41; 95 % CI 17.27–278.99), and grades B–C (OR 7.63), supporting active inflammation with inadequate plaque control as a non-definitional component of an unfavorable phenotype.

5. Given the small, exclusively male carrier group and the single-center, cross-sectional design, the findings are hypothesis-generating.

*Prospects for further research. Larger multicenter cohorts including both sexes should replicate these findings and externally validate the IIHI with clinical thresholds; longitudinal studies accounting for tobacco exposure, subgingival microbiota, and psychoemotional status should determine whether the combined recessive TLR4 profile and the IIHI predict disease progression and treatment outcomes and can guide individualized prevention and therapy.*

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**Conflict of interest.** The authors have no conflicts of interest to declare.

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