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TNF-α −308G/A (rs1800629) polymorphism and susceptibility to anogenital warts and their recurrence in Uzbek women from the Aral Sea Basin: a preliminary case–control study



Sh.A. Yusupova
Urganch davlat tibbiyot instituti. V.b dotsent. PhD
Email: shahnoza.yusupova90@gmail.com
ORCID number: https://orcid.org/0000-0002-6889-3701
Telefon raqami: +998 97 211 9333.

Ш.А.Юсупова
Ургенчский государственный медицинский институт. Доцент . PhD

Sh.A.Yusupova
Urgench State Medical Institute. Dotsent, Phd.

Abstract

Background: Anogenital warts caused by human papillomavirus follow a chronic, frequently relapsing course, and host immunogenetic factors may influence susceptibility and recurrence. The aim of this study was to examine the distribution of TNF-α rs1800629 (−308 G/A) alleles and genotypes in women with anogenital warts from the Aral Sea basin and to assess their possible association with disease susceptibility and recurrence.

The study material comprised venous blood samples obtained from 101 women with clinically confirmed anogenital warts (recurrent, n = 66; non-recurrent, n = 35) and 95 age- and ethnicity-matched healthy controls. Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP); allele and genotype frequencies were compared using the χ^2 test with calculation of odds ratios and 95% confidence intervals.

The results revealed a low frequency of the minor A allele in both groups (6.9% versus 5.8%) and complete absence of the A/A genotype. No statistically significant differences were observed between patients and controls (OR = 1.2; 95% CI: 0.53–2.86; p = 0.70) or between the recurrent and non-recurrent subgroups.

The obtained findings indicate the absence of a major independent contribution of TNF-α rs1800629 to susceptibility to anogenital warts and their recurrence in this population; the low minor-allele frequency limits statistical power, and confirmation in larger multicentre studies is required.

Keywords: anogenital warts, human papillomavirus, TNF-α, rs1800629, gene polymorphism, recurrence, Uzbekistan.

TNF-α −308G/A (rs1800629) polimorfizmi va Orol dengizi havzasidagi o‘zbek ayollarida anogenital so‘gallarga moyillik hamda ularning retsidivlanishi: dastlabki «kasal–nazorat» tadqiqoti

ANNOTATSIYA

Dolzarbligi. Odam papillomavirusi qo‘zg‘atadigan anogenital so‘gallar surunkali va tez-tez retsidivlanuvchi kechish bilan tavsiflanadi, xo‘jayin organizmining immunogenetik omillari esa kasallikka moyillik va retsidivlanish ehtimoliga ta’sir ko‘rsatishi mumkin. Tadqiqotning maqsadi Orol dengizi havzasida yashovchi anogenital so‘gallarga chalingan ayollarda TNF-α rs1800629 (−308 G/A) allellari va genotiplari taqsimotini o‘rganish hamda ularning kasallikka moyillik va retsidivlanish bilan mumkin bo‘lgan aloqasini baholashdan iborat bo‘ldi.

Tadqiqot materiali sifatida klinik jihatdan tasdiqlangan anogenital so‘gallari bo‘lgan 101 nafar ayoldan (retsidivlanuvchi, n = 66; retsidivlanmagan, n = 35) hamda yosh va etnik jihatdan moslashtirilgan 95 nafar sog‘lom ayoldan olingan vena qoni namunalari xizmat qildi. Genotiplash polimeraza zanjir reaksiyasi – restriksion fragmentlar uzunligi polimorfizmi (PZR-RFUP) usulida bajarildi; allel va genotip chastotalari χ^2 mezoni yordamida taqqoslanib, shanslar nisbati va 95 % ishonch oralig‘i hisoblandi.

Natijalar har ikkala guruhda ham minor A allelining past chastotada uchrashini (6,9 % va 5,8 %) hamda A/A genotipining umuman aniqlanmasligini ko‘rsatdi. Bemorlar va nazorat guruhi o‘rtasida (SN = 1,2; 95 % II: 0,53–2,86; p = 0,70), shuningdek retsidivlanuvchi va retsidivlanmagan kichik guruhlar o‘rtasida statistik jihatdan ahamiyatli farq aniqlanmadi.

Olingan ma’lumotlar mazkur populyatsiyada TNF-α rs1800629 polimorfizmining anogenital so‘gallarga moyillik va ularning retsidivlanishiga sezilarli mustaqil hissa qo‘shmasligidan dalolat beradi; minor allelning past chastotasi statistik quvvatni cheklaydi va natijalar yirikroq ko‘p markazli tadqiqotlarda tasdiqlanishini talab qiladi.

Kalit so‘zlar: anogenital so‘gallar, odam papillomavirusi, TNF-α, rs1800629, gen polimorfizmi, retsidiv, O‘zbekiston.

Полиморфизм TNF-α −308G/A (rs1800629) и предрасположенность к аногенитальным кондиломам и их рецидивированию у узбекских женщин Приаралья: предварительное исследование «случай–контроль»

АННОТАЦИЯ

Актуальность. Аногенитальные кондиломы, вызываемые вирусом папилломы человека, характеризуются хроническим, часто рецидивирующим течением, а иммуногенетические факторы хозяина могут влиять на предрасположенность к заболеванию и вероятность рецидива. Целью настоящего исследования явилось изучение распределения аллелей и генотипов TNF-α rs1800629 (−308 G/A) у женщин с аногенитальными кондиломами, проживающих в Приаралье, и оценка их возможной связи с предрасположенностью к заболеванию и его рецидивированием.

Материалом исследования послужили образцы венозной крови 101 женщины с клинически подтверждёнными аногенитальными кондиломами (с рецидивами, n = 66; без рецидивов, n = 35) и 95 здоровых женщин, сопоставимых по возрасту и этнической принадлежности. Генотипирование выполнено методом полимеразной цепной реакции с анализом полиморфизма длин рестрикционных фрагментов (ПЦР-ПДРФ); частоты аллелей и генотипов сравнивали с помощью критерия χ^2 с расчётом отношения шансов и 95 % доверительного интервала.

Результаты показали низкую частоту минорного аллеля A в обеих группах (6,9 % против 5,8 %) и полное отсутствие генотипа A/A. Статистически значимых различий между больными и контролем (ОШ = 1,2; 95 % ДИ: 0,53–2,86; p = 0,70), а также между подгруппами с рецидивами и без них не выявлено.

Полученные данные свидетельствуют об отсутствии существенного самостоятельного вклада полиморфизма TNF-α rs1800629 в предрасположенность к аногенитальным кондиломам и их рецидивирование в данной популяции; низкая частота минорного аллеля ограничивает статистическую мощность, что требует подтверждения результатов в более крупных многоцентровых исследованиях.

Ключевые слова: аногенитальные кондиломы, вирус папилломы человека, TNF-α, rs1800629, полиморфизм гена, рецидив, Узбекистан.

Introduction

Anogenital warts (AGWs) are among the most common clinical manifestations of human papillomavirus (HPV) infection and constitute a substantial public-health and quality-of-life burden [1]. Low-risk HPV genotypes, particularly types 6 and 11, are responsible for the majority of AGWs, yet the clinical course varies considerably between individuals, ranging from spontaneous regression to persistent and frequently recurrent disease. Despite the availability of ablative and

immunomodulatory treatments, post-treatment recurrence rates remain high — reported in the range of approximately 20–50% — which suggests that host-related factors, including genetic predisposition, contribute to disease persistence and recurrence. Although prophylactic HPV vaccination has substantially reduced the burden of HPV-related disease [2–5], AGWs remain common, particularly among unvaccinated populations, and their management continues to be complicated by frequent recurrence.

The immune response to HPV is mediated by both innate and adaptive mechanisms, in which cytokines play a central regulatory role [6]. Host immune competence strongly influences HPV persistence and clearance [7], and genetic variation affecting immune regulation and the local immune microenvironment may modulate individual susceptibility to infection and its clinical outcomes [8]. Among the cytokines involved in antiviral defence, tumour necrosis factor-alpha (TNF-α) is a key pro-inflammatory mediator that promotes inflammation, activates immune effector cells, and contributes to apoptosis of virus-infected cells.

The gene encoding TNF-α is located on chromosome 6p21.3 within the major histocompatibility complex (MHC) region, a locus of extensive relevance to immune-mediated and infectious diseases. Several single-nucleotide polymorphisms in the TNF-α promoter modulate gene transcription, and the rs1800629 variant (−308 G/A) is among the most extensively studied. The minor A (−308A) allele has been associated with increased transcriptional activity and enhanced TNF-α production compared with the common G allele, although functional studies have yielded inconsistent results; such variation could in principle influence the intensity of the inflammatory and antiviral response to HPV.

TNF-α promoter polymorphisms, including rs1800629, have been investigated in relation to susceptibility to viral infections, autoimmune disorders, and several malignancies, including HPV-associated cervical carcinogenesis [9,10]. However, reported findings have been inconsistent, and marked differences in allele frequencies and effect estimates have been observed between populations [9,10]. Moreover, most available data derive from studies of high-risk HPV and cervical neoplasia rather than benign low-risk HPV-driven lesions such as anogenital warts, and the relevance of these findings to AGWs remains uncertain. Additional co-factors — including co-infections that may enhance HPV persistence — further complicate the host–virus relationship [11].

To date, no data are available on the TNF-α rs1800629 polymorphism in relation to anogenital warts in Central Asian populations, including Uzbekistan. Therefore, the present study aimed to explore preliminary associations between TNF-α rs1800629 and susceptibility to anogenital warts

and their recurrence in women from Uzbekistan (Aral Sea basin), a population that has not previously been characterised in this context. Given the exploratory design and limited sample size, the findings are intended to inform hypotheses for confirmatory studies rather than to establish a clinically actionable genetic marker.

Methods

Ethics statement

This study was approved by the Ethics Committee of Urgench State Medical Institute (Protocol No. 7, dated March 15, 2023) and conducted in accordance with the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants prior to enrollment.

Study design and population

A case-control study was conducted at Urgench State Medical Institute between April 2023 and December 2024. The case group comprised 101 women aged 18–45 years with clinically confirmed anogenital warts. The control group consisted of 95 healthy women without a history of HPV-associated disease, matched to cases by age and ethnicity (Uzbek).

Inclusion criteria for cases: female sex; age 18–45 years; a clinically confirmed diagnosis of anogenital warts with a positive HPV DNA test; and willingness to provide written informed consent.

Exclusion criteria: immunodeficiency disorders; autoimmune diseases; malignancies; pregnancy; and chronic systemic diseases.

Clinical course classification and follow-up

Following standard treatment of visible lesions, patients were monitored for recurrence. Recurrent anogenital warts (n = 66) were defined as the reappearance of typical lesions after documented complete clinical resolution, occurring within a standardized 6-month post-treatment observation window. Non-recurrent anogenital warts (n = 35) were defined as the absence of any recurrence throughout the same 6-month window. Because the minimum follow-up was 6 months for every participant, both subgroups were classified over an identical observation period, avoiding differential misclassification between groups; patients were additionally monitored up to 12 months.

All patients underwent standard ablative treatment (CO_2 ablation) of visible lesions according to institutional protocol, and were scheduled for clinical follow-up at 1, 3, 6, and 12 months after complete clinical resolution. The total observation period was 12 months, and all patients had a minimum follow-up of 6 months.

HPV detection

Specimen collection.

Epithelial material was obtained directly from the anogenital wart lesions (condylomata acuminata) by scraping the surface of representative warts, and used as the material for PCR-based diagnostics.

Assay principle.

HPV DNA was detected and the low-risk genotypes differentiated using the AmpliSens® HPV 6/11-FL kit (Central Research Institute of Epidemiology, Moscow, Russia), based on the simultaneous amplification of HPV DNA target regions together with a fragment of the human β-globin gene, which served as an endogenous internal control (IC), followed by endpoint hybridization–fluorescence detection (FEP format).

Procedure.

The analysis comprised: (i) extraction of DNA from the biological specimen; (ii) amplification of the target region with endpoint hybridization–fluorescence detection (FEP format); and (iii) analysis and interpretation of the results.

Detection and interpretation.

Fluorescence signal intensity was recorded on an ALA-1/4 fluorimeter (BIOSAN, Riga, Latvia) across three channels: the FAM channel detected accumulation of the amplification product of HPV genotype 6, the HEX channel detected HPV genotype 11, and the ROX channel detected the internal-control (β-globin) DNA. Genotypes were called from the level of the fluorescence signal relative to background in the corresponding channels, referenced to the kit's control samples, and interpreted automatically by the ALA-1 software. Specimens positive in both the HPV 6 and HPV 11 channels were classified as mixed HPV 6/11 infections.

DNA extraction

Peripheral venous blood (5 mL) was collected from all participants into dry plastic tubes containing 0.5 mL of 0.5 M EDTA (pH 7.8). Genomic DNA was extracted from lymphocyte nuclei using an in-house optimised salting-out protocol. Briefly, 500 µL of whole blood was mixed with a lysis buffer containing 0.32 M sucrose, 10 mM Tris-HCl (Sigma, USA; pH 7.5), 5 mM $MgCl_2$, and 1% Triton X-100 (Sigma X-100). The mixture was centrifuged at 12,000 rpm and the supernatant was collected, to which a lysis solution containing 1 M Tris-HCl (pH 7.5), 0.5 mM EDTA (pH 8.0; Sigma, USA), 0.5% SDS (Sigma, USA), and 100 µg/mL proteinase K was added. Samples were incubated for 1 h at 56 °C; 100 µL of 6 M NaCl was then added, and the tubes were vortexed vigorously for 15–20 s and centrifuged at 12,000 rpm for 5 min. The DNA-containing supernatant

was transferred to a sterile tube, and 40 µL of 3 M sodium acetate and 800 µL of cooled 96% ethanol were added, followed by mixing and centrifugation at 14,000 rpm for 15 min. The resulting DNA precipitate was washed with 1.0 mL of 70% ethanol and centrifuged; the pellet was air-dried and dissolved in 50–150 µL of TE buffer or deionised water. DNA concentration and purity were assessed spectrophotometrically (NanoDrop 2000, USA) by measuring absorbance at 260 and 280 nm, with an A260/A280 ratio of approximately 1.8 considered acceptable. Extracted DNA was stored in TE buffer at +4 °C.

TNF-α rs1800629 genotyping

Genotyping of the TNF-α rs1800629 (−308 G/A) polymorphism was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The oligonucleotide primers for the target polymorphism were designed and optimised using Oligo v.6.31 software (Molecular Biology Insights Inc., USA) and synthesised by Litech (Moscow, Russia). The following primers were used to amplify the TNF-α promoter region containing rs1800629:

Forward: 5′-AATAGGTTTTGAGGGCCATG-3′

Reverse: 5′-ATCTGGAGGAAGCGGTAGTG-3′

PCR was performed in a 25-µL reaction volume containing 50–100 ng of genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM of each primer, and 1 U Taq DNA polymerase. Thermocycling conditions were: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 7 min.

The 222-bp PCR product was digested with the NcoI restriction enzyme at 37 °C for 1 h. In this assay, the −308G allele generates an NcoI recognition site (CCATGG) and is cleaved into two fragments (206 bp and 16 bp), whereas the −308A allele lacks the recognition site and remains uncut (222 bp). Digestion products were resolved on a 3% agarose gel stained with ethidium bromide and visualised under UV light; the 16-bp fragment migrates off the gel and is not routinely visualised, so on electrophoresis the −308G allele appears as a single 206-bp band, the −308A allele as a single 222-bp band, and the heterozygous G/A genotype as two bands (222 bp and 206 bp).

For quality control, 10% of samples (n = 20) were randomly selected and re-genotyped independently, with 100% concordance observed. Positive and negative controls were included in each PCR run.

Statistical analysis

Allele and genotype frequencies were determined by direct counting. Hardy–Weinberg equilibrium (HWE) in the control group was assessed using an exact test, given the low expected

count of the minor-allele homozygote. Statistical comparisons between groups were performed using Pearson's chi-square test or Fisher's exact test (when expected cell counts were <5). Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess associations between the TNF-α rs1800629 polymorphism and disease risk. In addition to comparisons of recurrent and non-recurrent subgroups with controls, a direct comparison of genotype frequencies between recurrent and non-recurrent patients was performed to evaluate the independent contribution of the polymorphism to recurrence. Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as $p < 0.05$ (two-tailed). Given the limited sample size and the small number of A-allele carriers, p-values close to the threshold should be interpreted with caution.

Results

Demographic and clinical characteristics

The demographic and clinical characteristics of the study participants are presented in Table 1. The mean age was 28.4 ± 6.2 years in the case group and 27.9 ± 5.8 years in the control group ($p = 0.56$). All participants were of Uzbek ethnicity. Among the 101 patients with anogenital warts, 35 (34.7%) had non-recurrent disease and 66 (65.3%) had recurrent disease. HPV type 6 was the most frequently detected genotype (58.4%), followed by HPV type 11 (31.7%) and mixed HPV 6/11 co-detection (9.9%).

Table 1. *Demographic and clinical characteristics of study participants.*

Characteristic	Cases (n = 101)	Controls (n = 95)	p-value
Age (years, mean ± SD)	28.4 ± 6.2	27.9 ± 5.8	0.56
Ethnicity (Uzbek), n (%)	101 (100%)	95 (100%)	—
Non-recurrent AGW, n (%)	35 (34.7%)	—	—
Recurrent AGW, n (%)	66 (65.3%)	—	—
HPV type 6, n (%)	59 (58.4%)	—	—

Characteristic	Cases (n = 101)	Controls (n = 95)	p-value
HPV type 11, n (%)	32 (31.7%)	—	—
Mixed HPV 6/11, n (%)	10 (9.9%)	—	—

AGW, anogenital warts; HPV, human papillomavirus; SD, standard deviation.

Hardy–Weinberg equilibrium

Because the expected count of the A/A genotype was less than one, the genotype distribution in the control group was tested for Hardy–Weinberg equilibrium using an exact test. The observed genotype frequencies did not deviate significantly from Hardy–Weinberg equilibrium (exact test, $p \approx 1.00$).

Allele and genotype distribution

The distribution of TNF-α rs1800629 alleles and genotypes in patients and controls is presented in Table 2. The frequency of the A allele was similar in the case and control groups (6.9% vs 5.8%), and the A/A genotype was not observed in either group. No statistically significant differences were detected for either allele or genotype comparisons (all $p > 0.05$).

Table 2. *Distribution of TNF-α rs1800629 alleles and genotypes in patients with anogenital warts and controls.*

Marker	Cases, n (%)	Controls, n (%)	χ^2	OR (95% CI)	p-value
Alleles	**n = 202**	**n = 190**			
G	188 (93.1%)	179 (94.2%)	—	Reference	—
A	14 (6.9%)	11 (5.8%)	0.2	1.2 (0.54–2.74)	0.70
Genotypes	**n = 101**	**n = 95**			
G/G	87 (86.1%)	84 (88.4%)	—	Reference	—

Marker	Cases, n (%)	Controls, n (%)	χ^2	OR (95% CI)	p-value
G/A	14 (13.9%)	11 (11.6%)	0.2	1.2 (0.53–2.86)	0.70
A/A	0 (0.0%)	0 (0.0%)	—	Not estimable	—

OR, odds ratio; CI, confidence interval. The A/A genotype was not detected in any participant.

Subgroup analysis: recurrent and non-recurrent anogenital warts

A direct comparison of allele and genotype distributions between recurrent and non-recurrent AGW patients is presented in Table 3. Genotype frequencies were similar in the recurrent subgroup (G/A 15.2%) and the non-recurrent subgroup (G/A 11.4%) and comparable to those of controls (G/A 11.6%), with the A/A genotype absent from all groups. The direct recurrent-versus-non-recurrent comparison of TNF-α rs1800629 revealed no statistically significant difference in allele or genotype distribution (all $p > 0.05$), indicating that the present data do not support the polymorphism as an independent predictor of recurrence.

Table 3. *Direct comparison of TNF-α rs1800629 allele and genotype distributions between recurrent and non-recurrent anogenital warts.*

Allele / Genotype	Recurrent AGW (n = 66), n (%)	Non-recurrent AGW (n = 35), n (%)	χ^2	p	OR (95% CI)
Alleles	**n = 132**	**n = 70**			
G	122 (92.4%)	66 (94.3%)	—	—	Reference
A	10 (7.6%)	4 (5.7%)	0.2	0.70	1.35 (0.41–4.48)
Genotypes	**n = 66**	**n = 35**			

Allele / Genotype	Recurrent AGW (n = 66), n (%)	Non-recurrent AGW (n = 35), n (%)	χ^2	p	OR (95% CI)
G/G	56 (84.8%)	31 (88.6%)	—	—	Reference
G/A	10 (15.2%)	4 (11.4%)	0.3	0.70	1.38 (0.40–4.78)

χ^2 and p values compare recurrent versus non-recurrent AGW subgroups. The G allele and the G/G genotype were used as the reference categories. OR, odds ratio; CI, confidence interval. The A/A genotype was not observed in either subgroup. Because expected counts in the non-recurrent subgroup were <5, Fisher's exact test was also applied and yielded concordant, non-significant results (allele and genotype, p = 0.77).

Discussion

The present case–control study explored a possible association between the TNF-α rs1800629 (−308 G/A) promoter polymorphism and susceptibility to anogenital warts, including recurrent forms, in women from Uzbekistan. We found no statistically significant differences in allele or genotype frequencies between patients and controls, or between recurrent and non-recurrent subgroups. Notably, the minor A allele was infrequent in this population, and the A/A genotype was not observed in any participant. These findings suggest that TNF-α rs1800629 does not act as a major independent determinant of susceptibility to, or recurrence of, HPV-related anogenital warts in this cohort — although, as detailed below, limited statistical power constrains this interpretation.

TNF-α has a well-established role in antiviral immunity, promoting inflammation, activating immune effector cells, and inducing apoptosis of infected cells, and the −308A allele has been linked to increased TNF-α transcription. A biological rationale therefore exists for hypothesising that variation at this locus could influence the host response to HPV. However, HPV infection outcomes are polygenic and multifactorial: they are shaped by additional cytokine and immune-gene polymorphisms, viral genotype and load, and behavioural and environmental exposures, any of which may outweigh the contribution of a single promoter variant. The absence of an association in our data is consistent with this multifactorial model and may also reflect the low frequency of the A allele in this population, which limits the ability of a modest-sized study to detect a small effect.

Our results can be interpreted alongside previous studies of TNF-α polymorphisms in HPV-related disease, most of which have focused on cervical carcinogenesis rather than benign warts. A comprehensive analysis by Marzbanrad et al. examined the relationship between TNF-α rs1800629 and susceptibility to cervical cancer [9], and studies of combined TNF-α and IL-10 polymorphisms have reported population-specific associations with cervical carcinogenesis [10]. Cytokine-mediated immune responses are increasingly recognised as important modulators of HPV-related disease [6]. Across these reports, findings have been inconsistent, with some populations showing associations and others — including the present cohort — showing none. The scarcity of studies specifically addressing TNF-α polymorphisms in anogenital warts limits direct comparison, which is precisely why the present findings, even though null, add value in an underexplored area.

The low frequency of the −308A allele observed in our Uzbek cohort (5.8% in controls) is an important feature of these data. Population-specific differences in TNF-α promoter allele frequencies are well documented, and a low minor-allele frequency both reduces the statistical power to detect associations and may itself modulate the population-level relevance of the variant. This underscores the importance of conducting immunogenetic association studies across diverse ethnic groups, including previously uncharacterised Central Asian populations.

Strengths and limitations

The strengths of this study include a clearly defined case–control design with stratification by disease course, and the inclusion of an underrepresented Central Asian population, providing what is, to our knowledge, among the first data on TNF-α rs1800629 in relation to anogenital warts in this region.

Several limitations must be acknowledged and shape the conclusions that can be drawn. First, and most importantly, the low frequency of the A allele and the complete absence of the A/A genotype (0 of 196 participants) markedly limited statistical power; a non-significant result under these conditions may reflect a Type II error rather than a true absence of effect, and a modest association cannot be excluded. No formal a priori power calculation was performed, consistent with the exploratory nature of the study. Second, important behavioural, clinical, and immunological confounders — including smoking, number of sexual partners and condom use, prior HPV vaccination, treatment modality, lesion burden, and immune parameters — were not systematically collected and could not be incorporated into the analysis. Third, controls were classified as healthy on the basis of the absence of a history of HPV-associated disease but were not tested for subclinical HPV infection, so misclassification of some controls cannot be excluded. Fourth, recurrence was

defined using a 6-month post-treatment window, which may misclassify late recurrences occurring beyond this period. Fifth, HPV genotype and viral load were not integrated into the genetic analysis. Sixth, only a single SNP within the TNF-α promoter was examined; haplotype analysis, gene–gene interactions, and study of additional immune- and apoptosis-related polymorphisms would provide a more complete genetic picture. Seventh, although all participants were of Uzbek ethnicity, no formal assessment of population substructure (e.g., genomic control) was performed, and residual population stratification cannot be fully excluded. Finally, the study was conducted at a single centre in one region of Uzbekistan, limiting generalisability, and the cross-sectional nature of genotyping does not permit causal inference.

Conclusion

In this exploratory case–control study in women from Uzbekistan, the TNF-α rs1800629 (−308 G/A) polymorphism was not associated with susceptibility to anogenital warts or with disease recurrence. The minor A allele was infrequent and the A/A genotype was absent, so these findings most plausibly indicate a lack of a major independent effect in this population — while acknowledging that the limited statistical power means a modest true association cannot be ruled out. Accordingly, these results should be regarded as preliminary and hypothesis-generating. On the basis of the present data, TNF-α rs1800629 genotyping cannot be recommended for clinical risk stratification of patients with anogenital warts. Replication in larger, adequately powered, multi-centre cohorts — ideally combined with the study of additional immune-related genes and haplotypes and with comprehensive collection of viral, behavioural, and immunological covariates — is required before any clinical application can be considered.

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