PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

ASSOCIAÇÃO DA VARIAÇÃO DO GENE TSHB COM HIPERTIREOIDISMO EM MULHERES DA PROVÍNCIA DE MAYSAN

THE ASSOCIATION OF TSHB GENE VARIATION WITH HYPERTHYROIDISM IN WOMEN OF MAYSAN PROVINCE

ارتباط التغاير في جين TSHB بفرط نشاط الغدة الدرقية لدى نساء محافظة ميسان

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RESUMO

Introdução: As disfunções da tireoide são condições complexas e poligênicas influenciadas por variantes genéticas. **Objetivo**: Avaliar, em caráter piloto e exploratório, a distribuição de uma substituição G>A observada na posição 113 do amplicon sequenciado do gene TSHB em mulheres iraquianas com hipertireoidismo. **Métodos**: Estudo caso-controle piloto com 15 mulheres com hipertireoidismo e 15 controles saudáveis, entre 18 e 45 anos. Um fragmento de 520 pares de bases do éxon 3 de TSHB foi amplificado por reação em cadeia da polimerase e sequenciado. As frequências genotípicas e alélicas foram descritas, e tabelas esparsas foram avaliadas pelo teste exato de Fisher. **Resultados**: As frequências dos genótipos GG, GA e AA foram 66,67%, 20,00% e 13,33% nos casos e 93,33%, 6,67% e 0% nos controles. O alelo A foi numericamente mais frequente nos casos (23,33% versus 3,33%). A análise assintótica produziu OR = 8,826 (IC95%: 1,012–76,963; P = 0,049), enquanto o teste exato de Fisher bilateral não atingiu significância estatística (P = 0,052). **Discussão**: A diferença observada constitui apenas uma tendência não significativa. O tamanho amostral reduzido, as células esparsas e os amplos intervalos de confiança limitam a precisão. O transcrito RefSeq revisado NM_000549.5 foi adotado como referência para a nomenclatura; contudo, o número 113 foi contado a partir do início do amplicon de 520 pb, e não a partir do códon de iniciação ATG. Portanto, essa posição é apresentada apenas como coordenada do amplicon e não como nomenclatura HGVS c. **Conclusões**: Os resultados são geradores de hipótese e não confirmam associação entre a variante e hipertireoidismo. Estudos multicêntricos maiores, com fenotipagem bioquímica detalhada e nomenclatura HGVS validada, são necessários.

Palavras-chave: *frequência alélica; variante genética; estudo caso-controle; hipertireoidismo; população iraquiana.*

ABSTRACT

Background: Thyroid disorders are complex polygenic conditions influenced by genetic variation. **Aim**: To conduct a pilot, exploratory assessment of a G>A substitution observed at position 113 of the sequenced TSHB amplicon in Iraqi women with hyperthyroidism. **Methods**: This pilot case–control study included 15 women with hyperthyroidism and 15 healthy controls aged 18–45 years. A 520-bp fragment of TSHB exon 3 was amplified by polymerase chain reaction and sequenced. Genotype and allele frequencies were described, and sparse contingency tables were evaluated using Fisher's exact test. **Results**: GG, GA, and AA genotype frequencies were 66.67%, 20.00%, and 13.33% in cases and 93.33%, 6.67%, and 0% in controls. The A allele was numerically more frequent in cases (23.33% vs. 3.33%). The asymptotic analysis yielded OR = 8.826 (95% CI: 1.012–76.963; P = 0.049), whereas the appropriate two-sided Fisher's exact test did not reach statistical significance (P = 0.052). **Discussion**: The observed difference represents a non-significant trend only. The small sample, sparse cells, and wide confidence interval substantially limit

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precision. The reviewed RefSeq transcript NM_000549.5 was selected as the reference sequence; however, position 113 was counted from the 5′ end of the 520-bp amplicon rather than from the transcript ATG start codon. It is therefore reported only as an amplicon coordinate and not as HGVS c. nomenclature. **Conclusions**: These hypothesis-generating findings do not establish an association between the variant and hyperthyroidism. Larger multicenter studies with detailed biochemical phenotyping and validated HGVS nomenclature are required.

***Keywords**: allele frequency; genetic variant; case–control study; hyperthyroidism; Iraqi population.*

الملخص

الخلفية: تُعد اضطرابات الغدة الدرقية حالات معقدة متعددة الجينات تتأثر بالتغايرات الوراثية. **الهدف**: إجراء تقييم استطلاعي أولي للاستبدال G>A المرصود في الموضع 113 من قطعة جين TSHB التي جرى تسلسلها لدى نساء عراقيات مصابات بفرط نشاط الغدة الدرقية. **طرائق العمل**: شملت دراسة استطلاعية للحالات والشواهد 15 امرأة مصابة بفرط نشاط الغدة الدرقية و15 امرأة سليمة تراوحت أعمارهن بين 18 و45 عاماً. ضُخمت قطعة بطول 520 زوجاً قاعدياً من الإكسون الثالث لجين TSHB بتفاعل البوليميراز المتسلسل ثم سُلْسِلَت. وُصفت تواترات الأنماط الجينية والأليلات، واستُخدم اختبار فيشر الدقيق للجداول ذات الخلايا قليلة العدد. **النتائج**: بلغت تواترات الأنماط GG وGA وAA لدى المصابات 66.67% و20.00% و13.33%، مقابل 93.33% و6.67% و0% لدى الشواهد. كان الأليل A أكثر تواتراً عددياً لدى المصابات (23.33% مقابل 3.33%). أعطى التحليل التقريبي OR = 8.826 (فاصل الثقة 95%: 1.012–76.963؛ P = 0.049)، في حين لم يبلغ اختبار فيشر الدقيق ثنائي الطرف مستوى الدلالة الإحصائية (P = 0.052). **المناقشة**: تمثل النتيجة اتجاهاً غير دال إحصائياً فقط، كما يحد صغر العينة وقلة أعداد بعض الخلايا واتساع فاصل الثقة من دقة التقدير. واعتمدت النسخة المرجعية المنقحة RefSeq NM_000549.5 مرجعًا للتسمية؛ إلا أن الموضع 113 حُسب ابتداءً من الطرف 5′ لقطعة التضخيم البالغ طولها 520 زوجًا قاعديًا، وليس من كودون البدء ATG في النسخة المرجعية. لذلك يُعرض هذا الموضع بوصفه إحداثيًا داخل قطعة التضخيم فقط، ولا يُعد تسمية HGVS من نوع c. **الاستنتاجات**: لا تثبت هذه النتائج الاستطلاعية وجود ارتباط بين المتغير وفرط نشاط الغدة الدرقية، وتستلزم دراسات متعددة المراكز بعينات أكبر وتوصيف كيميائي حيوي مفصل وتسمية HGVS موثقة.

الكلمات المفتاحية: *تواتر الأليل؛ متغير وراثي؛ دراسة الحالات والشواهد؛ فرط نشاط الغدة الدرقية؛ السكان العراقيون.*

1. INTRODUCTION

The thyroid gland is a major endocrine organ located in the anterior neck and composed of two lobes connected by an isthmus. Follicular cells synthesize triiodothyronine and thyroxine, whereas parafollicular cells produce calcitonin. Thyroid hormone production is regulated through the hypothalamic–pituitary–thyroid axis (Anandkumar *et al.*, 2020; Al-Suhaimi and Khan, 2022; Yao *et al.*, 2022).

Thyroid-stimulating hormone (TSH) is a heterodimeric glycoprotein composed of a common alpha subunit and a hormone-specific beta subunit encoded by TSHB. Circulating TSH is widely used in the biochemical evaluation of thyroid function, and binding of TSH to its receptor on thyroid follicular cells promotes thyroid hormone synthesis (Ushakov, 2024; Yang *et al.*, 2024).

The TSHB gene is located at chromosome 1p13.2 and contains three exons, of which exons 2 and 3 encode the mature protein. Pathogenic TSHB variants reported in the literature predominantly impair hormone synthesis, secretion, dimerization, or receptor activation and are therefore mainly associated with central hypothyroidism rather than hyperthyroidism (Nicholas *et al.*, 2016; Persani and Bonomi, 2017; Talebi *et al.*, 2020).

Accordingly, a direct causal mechanism linking a TSHB coding variant to hyperthyroidism has not been established. Hyperthyroidism is more commonly related to TSH-receptor autoantibodies or autonomous thyroid tissue. The present study was therefore designed as an exploratory association analysis and does not assume that the observed sequence change is causative.

The study specifically examined genotype and allele distributions for a G>A substitution observed at position 113 within the sequenced 520-bp amplicon. Because no reference-transcript accession was documented in the available laboratory record, this position is reported as an amplicon coordinate and should not be interpreted as validated HGVS coding-DNA nomenclature.

1.1. Aim

This pilot study aimed to explore whether the position-113 G>A substitution observed within the sequenced TSHB amplicon differed in frequency between Iraqi women with hyperthyroidism and healthy controls from Maysan Province.

2. MATERIALS AND METHODS

2.1. Study Population and Design

This pilot case–control study included 15 women with a clinical diagnosis of hyperthyroidism and 15 healthy controls aged

18–45 years. Participants were recruited in Maysan Governorate from 17 October 2024 to 25 May 2025, and blood samples were collected at Al-Sadr Hospital. The diagnosis recorded in the source dataset was based on hospital clinical and laboratory assessment; however, exact TSH/free-thyroxine thresholds, hyperthyroidism etiology, treatment status, and systematic exclusion criteria were not available for retrospective reporting. The sample size was determined by feasibility and available eligible participants; no a priori power calculation was performed. The study should therefore be interpreted as exploratory and hypothesis-generating.

2.2. Genomic DNA Extraction, PCR, and Genotyping

Genomic DNA was extracted from whole blood using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan). A 520-bp TSHB fragment was amplified with primers F: 5′-GGCTAAGCAATTCTTTCCCAGT-3′ and R: 5′-GCTCTCTAACGCCTGTGTAGG-3′ (Özhan et al., 2017). PCR was performed in 25 µL containing 3.5 µL DNA, 13 µL Master Mix, 1 µL of each primer, and 6.5 µL nuclease-free water. Cycling comprised 95°C for 5 min; 35 cycles of 95°C for 1 min, 55–58°C for 40 s, and 72°C for 45 s; and 72°C for 10 min. Products were separated on 1.5% agarose gel and visualized under ultraviolet light. Chromatograms were aligned and genotypes assigned using Geneious version 10.1.3. The reviewed human TSHB RefSeq transcript NM_000549.5 (protein accession NP_000540.2) was selected as the reference transcript for nomenclature review. The reported number 113 was counted from the 5′ end of the sequenced 520-bp PCR amplicon, not from the A of the transcript ATG initiation codon. Consequently, the observed change is reported as an amplicon-coordinate substitution (position-113 G>A) and not as HGVS c.113G>A.

2.3. Variant Nomenclature Verification

The reviewed reference transcript used for nomenclature assessment was NM_000549.5, with NP_000540.2 as the corresponding protein accession. HGVS coding-DNA numbering begins at the A of the ATG translation-initiation codon. Because the laboratory designation “position 113” was counted from the 5′ end of the 520-bp PCR amplicon, it is not valid HGVS c. notation. Therefore, the previous c.113G>A and p.Ala32Thr designations were withdrawn. The variant is described consistently throughout this manuscript as the position-113 G>A substitution within the sequenced TSHB amplicon. A definitive HGVS c. and p. designation can be assigned only after the original sequence files are aligned base-by-base to NM_000549.5 and the exact transcript coordinate is confirmed.

2.4. Statistical Analysis

Data were analyzed using SPSS version 26 and MedCalc version 20.0111. Genotype distributions were summarized descriptively. Hardy–Weinberg calculations are reported as exploratory because of the small sample. For the sparse 2×2 allele table, the two-sided Fisher’s exact test was used as the primary inferential test. The asymptotic Woolf/logit odds ratio and 95% confidence interval are retained as descriptive sensitivity estimates. Statistical significance was defined as $P < 0.05$. No adjustment for multiple comparisons was applied; therefore, all inferential findings are exploratory.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Identification of the Position-113 G>A Substitution

Multiple-sequence alignment of the sequenced TSHB amplicon revealed a G>A substitution at amplicon position 113 in women with hyperthyroidism (Figure 1). This coordinate is not presented as HGVS coding-DNA nomenclature because a reference transcript was not documented.

Sequence alignment revealed three genotypes at the studied site: GG, GA, and AA. A single yellow peak in the chromatogram indicated the wild-type genotype (GG), two overlapping red and yellow peaks indicated the heterozygous genotype (GA), and a single red peak corresponding to A indicated the homozygous variant genotype (AA) (Figure 2).

3.1.2. Genotype and Allele Frequencies

Three genotypes (GG, GA, and AA) were observed at the position-113 site. In the hyperthyroidism group, GG was the most frequent genotype (10/15; 66.67%), followed by GA (3/15; 20.00%) and AA (2/15; 13.33%). The corresponding control counts were 14, 1, and 0. Hardy–Weinberg calculations are shown descriptively in Table 1, but the small

group sizes limit their interpretation.

Genotype-level comparisons were not statistically significant. The A allele was numerically more frequent in cases than controls (7/30 [23.33%] vs. 1/30 [3.33%]). The asymptotic Woolf/logit analysis yielded OR = 8.826 (95% CI: 1.012–76.963; P = 0.049), but the two-sided Fisher's exact test, which is appropriate for the sparse allele table, did not reach the prespecified significance threshold (P = 0.052). The result is therefore reported as a non-significant trend and should be regarded as exploratory (Table 2).

3.1.3. Variant Annotation Status

The nucleotide substitution was detected reproducibly in the sequencing chromatograms. Nomenclature assessment used the reviewed TSHB transcript NM_000549.5; however, the reported position 113 originated from the amplicon coordinate system rather than from ATG-based HGVS coding-DNA numbering. Accordingly, the change is reported as position-113 G>A within the sequenced amplicon, and no definitive HGVS c. or p. designation or amino-acid consequence is claimed.

3.2. Discussion

The A allele was numerically more frequent among women with hyperthyroidism; however, Fisher's exact test yielded P = 0.052. Thus, the present data do not demonstrate a statistically significant association at α = 0.05. The direction and magnitude of the asymptotic odds ratio are unstable because the sample is small and one control cell contains only a single A allele.

The biological interpretation also requires caution. Published pathogenic TSHB variants primarily cause reduced TSH synthesis or function and central hypothyroidism, whereas hyperthyroidism is usually driven by TSH-receptor autoimmunity or autonomous thyroid tissue (Nicholas *et al*., 2016; Persani and Bonomi, 2017; Talebi *et al*., 2020). Therefore, the current observation cannot be interpreted through a simple loss-of-function mechanism, and a causal pathway linking this amplicon variant to hyperthyroidism remains unknown.

The reviewed RefSeq transcript NM_000549.5 was used to assess nomenclature. Because the original position 113 was counted from the start of the PCR amplicon rather than from the transcript ATG codon, the previous c.113G>A/p.Ala32Thr designation was not HGVS-compliant and was withdrawn. Functional interpretation should be reconsidered only after the original sequencing files are aligned to NM_000549.5, and a transcript-based c. and p. annotation is established.

The control-group allele frequency could not be validly benchmarked against gnomAD, dbSNP, or ClinVar without an unambiguous transcript/genomic coordinate and strand orientation. Database comparison, rsID assignment, and ClinVar status must therefore follow sequence re-annotation rather than be inferred from the current amplicon coordinate.

Overall, the study provides preliminary local genotype counts but not confirmatory evidence of association. Replication requires larger multicenter cohorts, predefined biochemical diagnostic criteria, documentation of Graves' disease or other etiologies, treatment-status data, exact statistical testing, transcript-based HGVS annotation, and subsequent functional assays only if a protein-altering variant is confirmed.

4. CONCLUSIONS

This pilot study identified a G>A substitution at position 113 of the sequenced TSHB amplicon. The A allele was numerically more frequent in women with hyperthyroidism than in controls (23.33% vs. 3.33%), but the appropriate two-sided Fisher's exact test did not reach statistical significance (P = 0.052). Consequently, the finding is hypothesis-generating and does not establish an association or causal relationship. Nomenclature review used the RefSeq transcript NM_000549.5 and confirmed that the laboratory number 113 was an amplicon coordinate, not an ATG-based HGVS c. coordinate; therefore, the prior c.113G>A/p.Ala32Thr designation and structural interpretation were withdrawn. Assignment of a definitive HGVS designation requires re-alignment of the original sequence files to NM_000549.5. Larger, independently replicated studies with detailed biochemical phenotyping and validated HGVS annotation are required.

5. DECLARATIONS

5.1. Study Limitations

The principal limitations are the pilot sample size, recruitment from one province, absence of an a priori power calculation, sparse genotype cells, wide confidence intervals, no correction for multiple comparisons, incomplete biochemical diagnostic thresholds and disease-etiology data, unavailable treatment and exclusion-criterion details, analysis of a single amplicon site, absence of the original sequence-file re-alignment needed to convert the amplicon coordinate to an NM_000549.5-based HGVS coordinate, and lack of functional validation. These limitations restrict precision, causal interpretation, reproducibility, and generalizability.

5.2. Acknowledgments

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5.3. Funding Sources

The authors funded this research.

5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw data are available upon reasonable request from the corresponding author, subject to participant confidentiality and ethical restrictions.

5.6. Author Contributions

Yamama A. Ogla: CD, DC, DAI, MW, FA. Salah H. Faraj: CD, DAI, CR, FA.

Code	English	Português
CD	Conception and Design	Concepção e Design
DC	Data Collection	Coleta de Dados
DAI	Data Analysis and Interpretation	Análise e Interpretação de Dados
MW	Manuscript Writing	Escrita do Manuscrito
CR	Critical Review	Revisão Crítica
FA	Final Approval	Aprovação Final

5.7. AI and Computational Tools Declaration

Tool: ChatGPT (OpenAI). Purpose: manuscript formatting, organization, and grammar/style improvement to comply with the journal template. All AI-assisted changes were reviewed and approved by the authors. No generative artificial intelligence was used for data fabrication, statistical testing, scientific interpretation, or independent scientific decision-making.

5.8. Research Integrity Declaration

The authors declare that this work is original, the methods were conducted ethically, and no data fabrication, results falsification, P-hacking, or selective reporting occurred.

5.9. Originality and Plagiarism Statement

This manuscript is original work, has not been previously published, is not under review elsewhere, and contains no unattributed copying. All sources are appropriately cited in APA format. The similarity index reported by the authors was 16%.

6. STUDIES INVOLVING HUMAN SUBJECTS

6.1. Ethics Committee Approval

The study involved human participants and was approved by the authorized ethics body of the College of Science, University of Misan (approval reference 756; 18 November 2024). The study complied with the Declaration of Helsinki. A digitized copy of the official approval letter must be uploaded as a separate supplementary submission file.

6.2. Informed Consent

Written informed consent was obtained from all participants before blood sample collection. Data were handled without personal identifiers to protect participant confidentiality and privacy.

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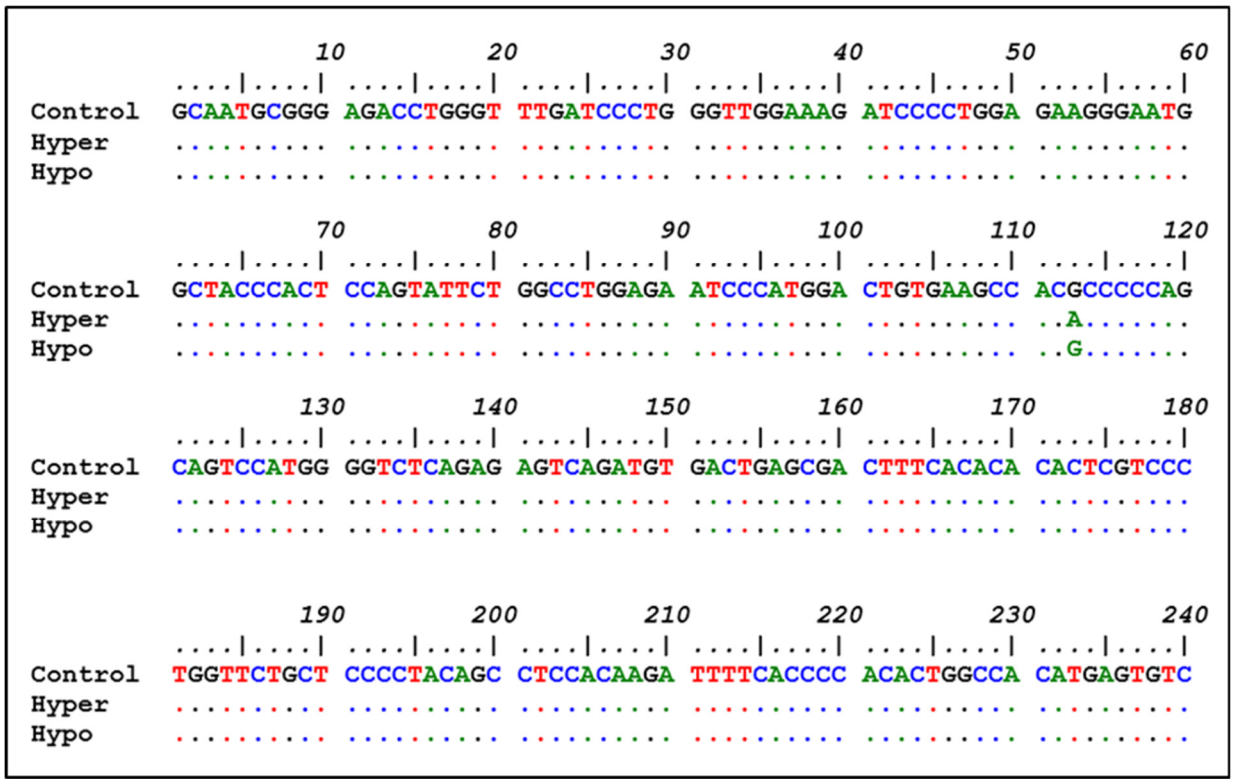

Figure 1. *Alignment of TSHB nucleotide sequences.*

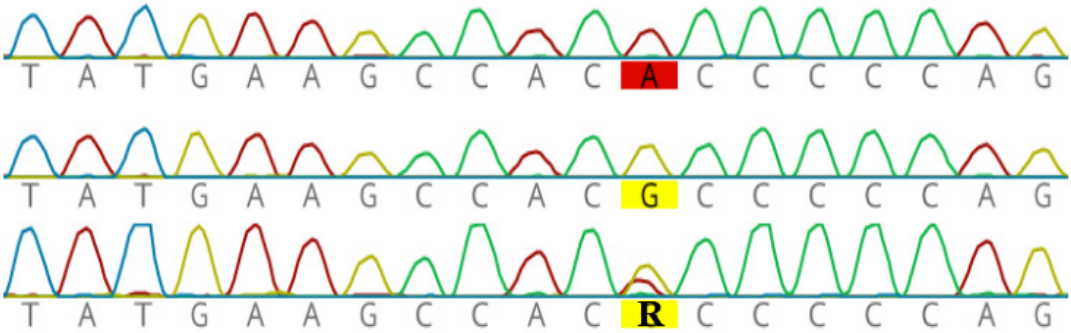

Figure 2. Sequencing chromatograms of the position-113 G>A substitution in the TSHB amplicon. The ambiguity code R indicates G/A.

Table 1. Observed and expected genotype counts at the position-113 G>A site in the hyperthyroidism and control groups

Group	Status	GG	GA	AA	χ^2	P-value
Cases	Observed	10	3	2	2.917	0.087
	Expected	8.82	5.37	0.82		
Control	Observed	14	1	0	0.017	0.893
	Expected	14.02	0.97	0.02		
Total observed		24	4	2		

HWE, Hardy–Weinberg equilibrium.

Table 2. Genotype and allele frequencies at the position-113 G>A site in the hyperthyroidism and control groups

Genotype/allele	Hyper n (%)	Control n (%)	OR	95% CI	P-value (Fisher exact where applicable)	PF/EF
GG	10 (66.67%)	14 (93.33%)	0.142	0.014–1.418	0.096	PF = 0.858
GA	3 (20.00%)	1 (6.67%)	3.500	0.320–38.233	0.304	EF = 0.714
AA	2 (13.33%)	0 (0.00%)	5.740	0.252–130.379	0.272	EF = 0.825
G	23 (76.67%)	29 (96.67%)	0.113	0.012–0.988	0.052	—
A	7 (23.33%)	1 (3.33%)	8.826	1.012–76.963	0.052	—

OR, odds ratio; CI, confidence interval; PF, preventive fraction; EF, etiological fraction. The allele-level P-value is the two-sided Fisher's exact result. Genotype-level estimates are exploratory; no multiple-comparison correction was applied.