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INVESTIGATION OF IFI16 GENE VARIANTS ASSOCIATED WITH IMMUNE RESPONSE IN THE VIETNAMESE POPULATION

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ABSTRACT

Objectives: *To determine the allele frequencies, genotype distribution and haplotype profiles of several variants within the promoter region of the IFI16 gene in the Vietnamese population.*

Subjects and methods: *A cross-sectional descriptive study was conducted on 132 peripheral blood samples collected from unrelated healthy Viet individuals at the Department of Biology and Medical Genetics, Military Medical University, between September 2025 and February 2026. Genomic DNA was extracted using the GeneJET™ kit; two target segments within the promoter region were amplified via Polymerase Chain Reaction (PCR) and analyzed using Sanger sequencing. Data processing and genetic analysis were performed using MEGA X, SPSS 20.0 and Arlequin 3.5 software.*

Results: *Six Single Nucleotide Polymorphisms (SNPs) were successfully genotyped: -1735A/C, -982A/G, -760G/T, -746A/G, -491A/C, and -224T/G. The Minor Allele Frequencies (MAFs) were determined to be 0.3410 (C), 0.0038 (G), 0.0909 (T), 0.3485 (G), 0.1250 (C), and 0.4242 (G), respectively. Haplotype analysis revealed 11 distinct combinations, among which AGAAG (42.0%), CGGAT (28.4%) and AGAAT (14.8%) were predominant, collectively accounting for over 85% of the total haplotype pool. Notably, the minor allele frequencies at rs3754466 and rs3754464 in the Viet cohort were significantly higher than those reported in European, African and American populations, while closely resembling East Asian populations.*

Conclusions: *This study establishes the first comprehensive genetic dataset regarding the IFI16 gene promoter region in the Viet population. The findings confirm distinct genetic divergence from Western cohorts and provide a baseline foundation for future case-control studies exploring genetic susceptibility to infectious and autoimmune disorders.*

Keywords: IFI16, promoter, SNP, haplotype, Vietnamese population, innate immunity, STING.

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1. INTRODUCTION

The innate immune system serves as the primary line of defense protecting the host against foreign pathogenic invasions and intracellular danger signals [1]. Within this defense mechanism, pattern recognition receptors (PRRs) play a pivotal role in detecting anomalous or foreign nucleic acids. Among these sensors, Interferon-inducible protein 16 (IFI16) has been identified as a critical intracellular DNA sensor capable of recognizing double-stranded DNA (dsDNA) derived from both bacterial and viral pathogens [2]. This structural recognition triggers the subsequent production of type I interferons (IFN-I) and proinflammatory cytokines via the STING (Stimulator of interferon genes) downstream signaling pathway [3].

Recent literature has further elucidated the expanded role of IFI16 in restricting both DNA and RNA viruses; furthermore, it enhances STING activation via a distinctive filament structure formed by its pyrin domain [4], [5]. Accumulating evidence underscores the multifunctional nature of IFI16, demonstrating its involvement not only in antiviral responses but also in gene expression regulation, cell cycle controls and its tight integration into the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus (SLE) [6], [7]. Specifically, elevated expression of IFI16 within renal tubular epithelial cells has been tightly correlated with macrophage infiltration during interstitial renal injury in patients with SLE [8].

Despite the clear biomedical significance of functional studies on IFI16, genetic variations-

particularly single nucleotide polymorphisms (SNPs) capable of modulating protein architecture or gene transcription efficiency-remain poorly characterized across different ethnic cohorts, leading to inter-individual variations in immune response [9]. Although several international consortia have mapped the allele and genotype frequencies of *IFI16* across European, American, and Northeast Asian populations, revealing profound inter-ethnic genetic diversity [10], data concerning Southeast Asian populations-especially the Viet population-remain remarkably scarce.

Due to a distinct anthropological evolutionary history and unique localized natural selection pressures, the distribution frequencies of immune-related genetic variants in the Viet population may harbor unique characteristics compared to other global reference populations [11]. The current lack of population-specific baseline data regarding the allele frequencies and haplotypes of the *IFI16* gene in Vietnam constitutes a critical knowledge gap, hindering accurate genetic risk assessments and the advancement of personalized therapeutic strategies for immune-mediated pathologies.

To address this limitation, the present study was designed under the hypothesis that the distribution frequencies of specific *IFI16* promoter variants associated with immune responses in the Viet population exhibit significant divergence from other global cohorts. The primary objective is to define the allele frequencies, genotype distributions and predominant haplotypes of representative *IFI16* polymorphisms within a healthy Viet cohort. The generated data will establish a foundational reference for population genetics and provide the groundwork for subsequent case-control association studies aimed at elucidating the relationship between these variants and host susceptibility to infectious and autoimmune diseases in Vietnam.

2. SUBJECTS AND METHODS

2.1. Subjects

A total of 132 archived peripheral blood samples obtained from unrelated healthy Viet individuals were utilized. The samples were maintained and analyzed at the Department of Biology and Medical Genetics, Military Medical Academy, covering the period from September 2025 to February 2026.

2.2. Methods

- Study design: A controlled, cross-sectional descriptive study design was deployed.

- Laboratory techniques:

+ DNA extraction: Genomic DNA was isolated using the GeneJET™ Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) in strict accordance with the manufacturer's instructions. The concentration and purity of the extracted DNA were verified via spectrophotometric quantification.

- Primer design: To investigate variants localized within the *IFI16* promoter region that potentially modulate gene expression efficiency, two specific primer pairs were designed to amplify the target promoter segments. The customized oligonucleotide sequences are detailed in Table:

- PCR Amplification: Polymerase chain reactions were conducted on a thermal cycler using an optimized amplification profile consisting of 35 cycles: initial denaturation followed by cycling steps of denaturation at 95°C, primer annealing at 58°C and elongation at 72°C. The resulting PCR amplicons were resolved via 2% agarose gel electrophoresis to confirm size specificity against a DNA molecular weight ladder.

Primer Name	Primer Sequence (5' - 3')	Target size
Pro.F1	5'-GGAAAATGAGGCCAGAAAGG-3'	1024 bp
Pro.R1	5'-CCGGTAGTGTGAGGAGTCTG-3'	
Pro.F2	5'-GGGGTTACAGCACGCTAAAGGGC-3'	1042 bp
Pro.R2	5'-GCTGACTAGTGCTGGCTTGCTCC-3'	

- Sanger sequencing: Following enzymatic purification, direct bidirectional Sanger sequencing of the verified PCR products was executed utilizing the SeqStudio 24 Sanger Sequencing System.

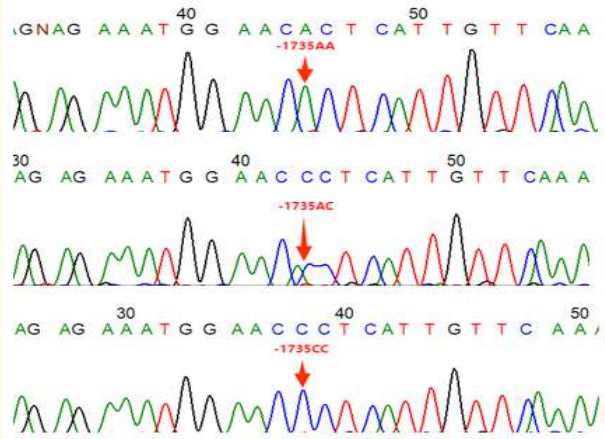
- Ethical considerations: This study received formal approval from the Institutional Review Board and Ethics Committee of the Military Medical Academy under Decision No. 5657/QĐ-HVQY, dated December 3, 2025. Strict data de-identification protocols were implemented to maintain participant confidentiality.

- Statistical analysis: Phenotypic and descriptive data processing was conducted using SPSS 20.0 software. The acquired DNA sequence chromatograms were aligned and analyzed against the canonical *IFI16* reference sequence deposited in GenBank using MEGA X software. Population genetics parameters, including allele frequencies, genotype distributions and conformity to Hardy-Weinberg Equilibrium (HWE), were calculated via Arlequin 3.5 software.

3. RESULTS

The investigation successfully mapped six polymorphic sites localized within the promoter region of the *IFI16* gene.

3.1. Allele and Genotype Frequencies at the -1735A/C

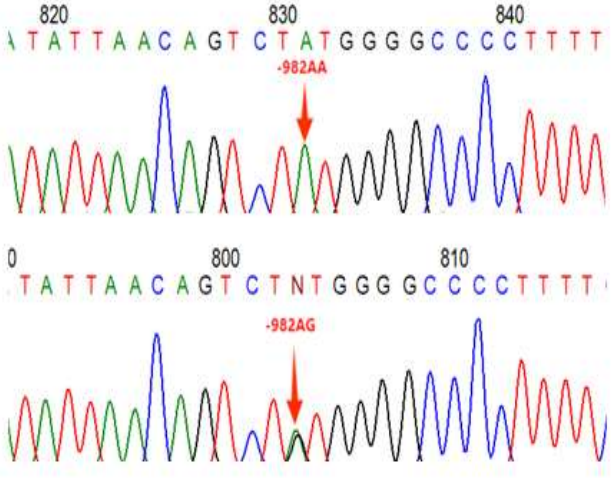


-1735A/C (rs3754466)	N=132
AA	60
AC	54
CC	18
pA	0,6590 (65,90%)
qC	0,3410 (34,10%)
P-HWE	0,302

Figure 1. Allele and genotype frequencies at the -1735A/C.

Sanger sequencing analysis at the -1735A/C locus demonstrated the clear segregation of all three possible genotypes within the study cohort (Figure 1). Among the 132 analyzed samples, the homozygous wild-type AA genotype was predominant (n = 60), followed by the heterozygous AC genotype (n = 54), whereas the homozygous mutant CC genotype exhibited the lowest frequency (n = 18). The calculated frequency for the ancestral major allele A was 65.90%, while the minor allele C stood at 34.10%. The observed genotype distribution conformed strictly to the Hardy-Weinberg Equilibrium -HWE = 0.302 > 0.05), validating the unbiased representativeness of the cohort sample relative to the general population.

3.2. Allele and Genotype Frequencies at the -982A/G

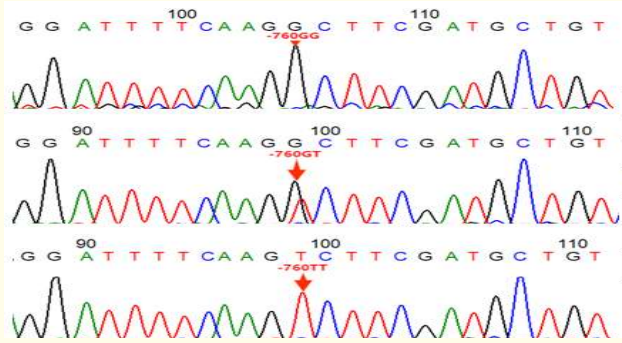


-982A/G (rs76575807)	N=132
AA	131
AG	1
GG	0
pA	0,9962 (99,62%)
qG	0,0038 (0,38%)
P-HWE	0,960

Figure 2. Allele and genotype frequencies at the -982A/G.

At the -982A/G polymorphic site, the sequencing tracks revealed an exceptionally high degree of evolutionary conservation within this genomic block among Viet individuals (Figure 2). Specifically, 131 out of 132 subjects carried the fixed homozygous AA genotype, with only a solitary instance of the heterozygous AG genotype recorded (n = 1) and a complete absence of the homozygous mutant GG genotype (n = 0). Consequently, the minor allele G frequency was extremely low (0.38%). Despite this skewed distribution, the locus remained within genetic equilibrium, demonstrating a high HWE value of 0.960.

3.3. Allele and Genotype Frequencies at the -760G/T

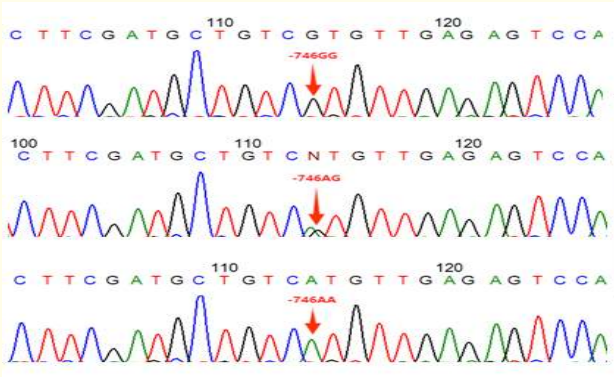


-760G/T(rs1465175)	N= 132
GG	109
GT	22
TT	1
pG	0,9091(90,91%)
qT	0,0909 (9,09%)
P-HWE	0,924

Figure 3: Allele and genotype frequencies at the -760G/T.

Genotypic screening at the -760G/T locus demonstrated that a vast majority of the subjects possessed the homozygous GG genotype (n = 109) (Figure 3). Genotypes harboring the minor alternative T allele occurred at substantially lower frequencies, with 22 subjects presenting as heterozygous GT and only a single subject identified as homozygous mutant TT (n = 1). The calculated allele frequencies for G and T were 90.91% and 9.09%, respectively. Hardy-Weinberg testing yielded a p-value of 0.924, confirming that the genotype architecture at this locus is highly stable within the evaluated population.

3.4. Allele and Genotype Frequencies at the -746A/G

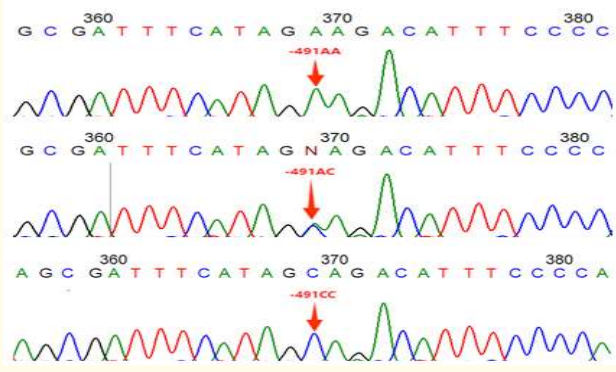


-746A/G (rs3754464)	N=134
AA	59
AG	54
GG	19
qA	0,6515 (65,15%)
qG	0,3485 (34,85%)
P-HWE	0,255

Figure 4: Allele and genotype frequencies at the -746A/G

The -746A/G locus exhibited a high degree of genetic polymorphism, mirroring the distribution pattern seen at the -1735 site (Figure 4). The homozygous AA genotype was detected most frequently (n = **59)**, followed closely by the heterozygous AG genotype (n = 54) and the homozygous GG genotype (n = 19). The minor allele G frequency reached 34.85%. Backed by a -HWE value of 0.255, the genotype configurations at this locus indicate that the population is in a state of genetic equilibrium, showing no significant evidence of disruptive selective pressures or genetic drift.

3.5. Allele and Genotype Frequencies at the -491A/C

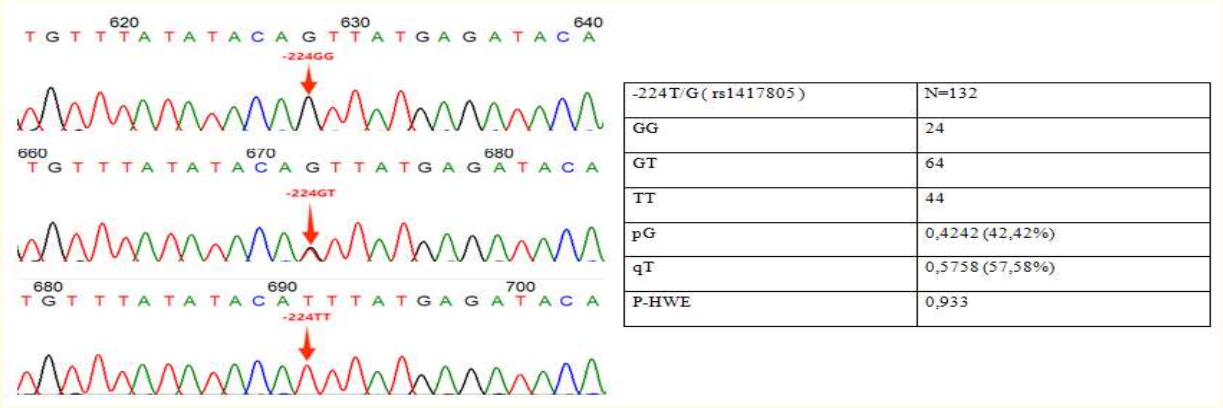


-491A/C (rs1417806)	N=132
AA	102
AC	27
CC	3
pA	0,875 (87,50%)
qC	0,125 (12,50%)
P-HWE	0,454

Figure 5: Allele and genotype frequencies at the -491A/C

Sequence chromatogram analysis at the -491A/C position indicated that the homozygous AA genotype comprised an overwhelming majority of the cohort, accounting for 102 out of 132 samples (Figure 5). In contrast, the heterozygous AC genotype was observed in 27 samples, while the homozygous CC genotype was confirmed in only 3 samples. The minor allele C frequency was computed at 12.50%. The distribution pattern showed no deviation from the Hardy-Weinberg Equilibrium, yielding a p-value of 0.454.

3.6. Allele and Genotype Frequencies at the -224T/G



-224T/G (rs1417805)	N=132
GG	24
GT	64
TT	44
pG	0,4242 (42,42%)
qT	0,5758 (57,58%)
P-HWE	0,933

Figure 6: Allele and genotype frequencies at the -224T/G.

The -224T/G variant recorded the highest level of heterozygosity across all six loci evaluated (Figure 6). The heterozygous GT genotype constituted the majority of the samples (n = 64), outnumbering both the homozygous TT (n = 44) and homozygous GG (n = 24) configurations. The calculated frequency of the alternative G allele was notably elevated at 42.42%. An HWE test value of 0.933 confirmed that the distribution of these alleles aligns precisely with classical population genetics models.

3.7. Comparative Analysis of Minor Allele Frequencies (MAF) and Haplotype Architectures

Table 1. Cross-population comparative analysis of Minor Allele Frequencies (MAF) for *IFI16* promoter variants

Polymorphism	dbSNP ID	Present Study	Europe	Africa	America	South Asia	East Asia	Japan	Korea
-1735A/C	rs3754466	0,3410	0,02149	0,07478	0,01844	0,2003	0,2576	0,21976	0,2140
-982A/G	rs76575807	0,0038	0.00001	0.00000	0.00000	0.0000	0,0079	0,01329	0,0135
-760G/T	rs1465175	0,0909	0,3360	0,2383	0,202	0,242	0,1359	0,26170	0,1816
-746A/G	rs3754464	0,3485	0,0159	0,1762	0,023	0,213	0,2788	0,22230	0,2014
-491A/C	rs1417806	0,1250	0,30169	0,26609	0,24977	0,2315	0,1668	0,26988	0,1947
-224T/G	rs1417805	0,4242	0,48733	0,09621	0,46120	0,3951	0,4631	0.43953	0,5073

Profound ethnic variations were noted at the -1735A/C (rs3754466) locus, where the frequency of the alternative C allele in the Viet cohort (0.3410) was drastically higher than those documented in European (0.0215), African (0.0748) and American (0.0184) cohorts. Similarly, at the -746A/G (rs3754464) site, the minor G allele frequency (0.3485) markedly outpaced Western populations. Conversely, at the -760G/T and -491A/C positions, the MAFs of the Viet cohort were noticeably lower than the global references. Broadly, the genetic profile of the *IFI16* promoter region in the Viet population exhibits a clear affinity toward East Asian populations (Japan, Korea) while maintaining localized variations.

Table 2. Haplotype frequencies and percentage distributions across the *IFI16* promoter block

No.	Haplotypes (-1735/-760/-746/-491/-224)	Haplotype Count	Percentage (%)
1	AGAAG	111	42.0
2	CGGAT	75	28.4
3	AGAAT	39	14.8
4	ATACT	17	6.4
5	CGGCT	8	3.0
6	CTGCT	5	1.9

No.	Haplotypes (-1735/-760/-746/-491/-224)	Haplotype Count	Percentage (%)
7	AGACT	3	1.1
8	AGGAT	3	1.1
9	ATAAT	1	0.4
10	CGAAG	1	0.4
11	CTGAT	1	0.4

The core haplotype configuration AGAAG was identified as the most dominant block, accounting for 42.0% of cases, followed closely by CGGAT (28.4%) and AGAAT (14.8%). Crucially, these three leading blocks comprise over 85% of the total genetic combinations, demonstrating high evolutionary stability within this promoter region. Rare configurations occurring below the 1% threshold may represent critical genetic signatures for subsequent disease-association tracks.

4. DISCUSSION

The rationale behind this study is rooted in the essential functional role of IFI16 as an intracellular DNA sensor that stimulates the STING cascade, driving the expression of type I interferons and essential inflammatory signals, alongside its roles in cell cycle checkpoints and transcription regulation [2],[3]. Recent structural biology studies confirmed that the specific filament-like organization of the IFI16 pyrin domain is critical for amplifying downstream STING activity [5]. Consequently, structural variations within the promoter region are highly likely to alter gene transcription levels, leading to significant variations in individual innate immune baselines [9].

Furthermore, clinical association studies have shown that point mutations within the *IFI16* locus significantly affect vascular wall inflammation metrics [12]. Although large-scale international genomic databases like the 1000 Genomes Project have mapped *IFI16* variations across Western and African cohorts [10], there has been a persistent data gap regarding Southeast Asian populations-specifically Vietnam, which features a distinct demographic history and unique regional selective pressures [11].

The current cross-sectional descriptive study of 132 healthy Viet subjects successfully profiled six promoter SNPs (-1735A/C, -982A/G, -760G/T, -746A/G, -491A/C and -224T/G). All target loci conformed to the Hardy-Weinberg equilibrium -HWE > 0.05), showing minor allele distributions of 0.3410 (C), 0.0038 (G), 0.0909 (T), 0.3485 (G), 0.1250 (C) and 0.4242 (G), respectively. Haplotype block evaluation resolved 11 core combinations, with three dominant signatures (AGAAG: 42.0%, CGGAT: 28.4% and AGAAT: 14.8%) covering more than 85% of the total haplotype pool, indicating structural stability in the population's genetic architecture.

Our comparative findings revealed striking differences when compared against Western reference datasets. At the -1735A/C (rs3754466) locus, the minor C allele frequency in Viet individuals (0.3410) is 16-fold higher than in European populations (0.0215), 4.5-fold higher than in African cohorts (0.0748) and 18-fold higher than in American datasets (0.0184) reported by the 1000 Genomes Project [10]. Similarly, the alternative G allele at the -746A/G (rs3754464) position showed a distinct increase compared to Western cohorts, whereas the frequencies at -760G/T and -491A/C were below global averages.

These baseline metrics closely resemble East Asian data profiles (Japan, Korea) while retaining distinct differences, aligning with whole-genome landscape mappings of the Viet population [11]. In addition, the -982A/G and -760G/T positions showed high conservation (MAF< 0.1), suggesting strong evolutionary conservation within these promoter regions.

From a mechanistic perspective, single nucleotide changes within promoter regions can alter the binding affinity of key transcription factor complexes (such as NF-κB or IRF families), thereby modulating downstream IFI16 expression levels [9]. Given that IFI16 binds foreign dsDNA to initiate STING-NF-κB signaling and induce type I IFNs [2], [3] and its proven involvement in vascular inflammation and hypoxic response pathways [13], the distinct allele frequencies observed in the Viet population may alter individual inflammatory baselines. This variation could help explain differences in susceptibility to specific infectious agents or autoimmune conditions, such as SLE, compared to Western populations [6], [7].

The predominant haplotypes identified, AGAAG and CGGAT, may act as protective or risk-conferring genetic signatures, a hypothesis that requires validation in future case-control studies. These findings hold significant translational value: they establish the first population-specific reference dataset for the *IFI16* gene in Vietnam, filling a critical research gap [11] and laying a foundation for

personalized medicine. These insights will enhance risk assessment strategies for infectious and autoimmune disorders, supporting the development of targeted prevention and treatment protocols tailored to the Viet population.

5. CONCLUSION

This study successfully mapped the allele frequencies, genotype distributions, and haplotype profiles of six key promoter variants of the *IFI16* gene within a cohort of 132 healthy Viet individuals. The experimental data validate our initial hypothesis: the frequencies of specific SNPs-notably rs3754466 and rs3754464 in the Viet population differ markedly from those in European, African, and American cohorts, showing significantly higher minor allele frequencies. Furthermore, the three dominant haplotypes-AGAAG (42.0%), CGGAT (28.4%), and AGAAT (14.8%)-, reflecting a stable and distinct genetic architecture in the Viet population.

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