Case Report

A variant in the TSH β gene resulted in discordant TSH levels in an Indian patient

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Keywords

TSH immunoassay interference, thyroid-stimulating hormone, TSHβ gene variant, epitope alteration, assay harmonization, Indian Patient

Abstract

A clinically euthyroid patient of Indian origin was identified with persistently undetectable TSH concentrations using our laboratory's third-generation Ultra TSH assay, raising concerns of assay interference. The discordant results, flagged by the treating physician, prompted an in-depth investigation to determine the cause of undetectable TSH values despite the patient's euthyroid clinical status. Over an eight-month period, three consecutive serum samples consistently showed TSH levels below 0.008 µIU/mL on our routine platform. To rule out analytical artifacts such as the high-dose hook (prozone) effect, heterophilic antibody interference, and other pre-analytical or analytical errors, the samples were re-evaluated under various dilution protocols and assay conditions. Reanalysis using two alternate FDA-approved TSH immunoassays (CLIA and ECLIA platforms) revealed a TSH concentration of 6.81 µIU/mL, consistent with the clinical picture and in stark contrast to our initial results. Given the persistence of this discrepancy and the suspected interference with antibody recognition, genetic analysis of the TSHB gene was performed. Sanger sequencing of the entire coding region revealed a homozygous A-to-G substitution (c.223A>G; AGA>GGA) in exon 3, resulting in an arginine-to-glycine amino acid change at codon 75 (R75G) in the mature TSH β-subunit (RefSeq: NP_000540.2). This variant was absent in a control subject of similar ethnic background with normal TSH levels on the same assay, supporting its role in the observed interference. The mutation likely alters the epitope conformation of the TSH molecule, reducing its recognition by monoclonal antibodies used in specific immunoassays without impairing its biological activity. This case underscores the importance of correlating laboratory results with clinical findings and highlights the need for cross-platform verification when discordant TSH values are encountered. Genetic variants affecting TSH structure can lead to misinterpretation of thyroid function, and efforts toward assay standardization and harmonization are essential to mitigate such diagnostic pitfalls.

Introduction

Thyroid-stimulating hormone (TSH) is a primary biomarker for evaluating thyroid function and is routinely measured using third-generation immunoassays due to their high sensitivity and analytical reliability. These assays, including chemiluminescent immunoassay (CLIA) and electrochemiluminescent immunoassay (ECLIA), typically employ monoclonal antibodies targeting epitopes on the TSH β-subunit. However, despite their robustness, these immunoassays are susceptible to analytical interference that can compromise accuracy. Such interference may arise from endogenous factors like heterophilic antibodies, high-dose hook effects, or more rarely, genetic variants that structurally modify the TSH molecule [1]. The clinical implications of platform diversity in immunoassay systems are multifaceted and significant for patient care. Immunoassay platforms utilize diverse antibodies, both polyclonal and monoclonal, designed by different manufacturers, with IVD validation typically occurring on specific populations, leading to generalized results that may not adequately account for global population diversity. This variability has profound patient impact, as genetic variants present significant clinical implications where patients may receive unnecessary treatment modifications despite having functionally normal TSH levels within established reference ranges, thereby emphasizing the critical importance of maintaining robust clinical-laboratory correlation in endocrine diagnostics. The key message from our findings is that such genetic variants, while relatively rare in occurrence, can lead to substantial diagnostic confusion and inappropriate clinical management if not properly recognized and addressed through comprehensive evaluation protocols. Immunoassay technology faces inherent limitations that challenge modern laboratory medicine, highlighting the urgent need for standardization and harmonization across analytical platforms. Until more harmonized assays or advanced techniques like mass spectrometry-offering greater specificity and less interference-are widely adopted, enhanced clinical-laboratory communication remains crucial. Our case study emphasizes the importance of vigilance among laboratory professionals and clinicians when results are discordant without clinical correlation, as such discrepancies may reflect analytical interferences rather than true pathology. Recognizing these interferences requires a systematic review of manufacturer instructions, considering factors like Human Anti-Mouse Antibodies (HAMA), Human Anti-Animal Antibodies (HAAA), prozone effects, biotin interference, and genetic variants such as the TSH-β R75G mutation that affect epitope recognition and cause platform-specific errors. This complex interference landscape underscores the need for clinical suspicion, cross-platform verification, and close collaboration between laboratory and clinical teams to ensure accurate diagnosis and optimal patient care. Variants that alter key amino acid residues within epitope regions can disrupt antigen-antibody binding, resulting in underestimation or non-detection of TSH levels. This form of interference is particularly concerning, as it may lead to misclassification of thyroid status, including the false diagnosis of hyperthyroidism. In some cases, TSH remains biologically active but is not immunoreactive in certain assay systems due to altered epitope conformation. These discrepancies emphasize the importance of assay design, particularly epitope selection, and the need for cross-platform verification when TSH results are incongruent with clinical findings. Furthermore, molecular genetic analysis can serve as a valuable tool to identify sequence variants in the TSHB gene that may affect assay performance. Awareness of these potential limitations and the implementation of harmonization strategies across assay platforms are critical for improving the diagnostic accuracy of TSH measurements in routine clinical practice [2], [3].

Case

A 41-year-old male resident of West Bengal, with a known history of carcinoma tongue status post-surgical resection and adjuvant radiotherapy completed two years prior, presented for routine follow-up. During longitudinal monitoring, a persistent and significant discrepancy in thyroid-stimulating hormone (TSH) levels was noted between our laboratory and an external reference laboratory, with both tests conducted within a one-week interval. Our laboratory consistently reported undetectable TSH levels (<0.008 µIU/mL) across three serum samples over an 8-month period using a third-generation ultra-sensitive immunoassay. In contrast, reanalysis of the same serum sample using an alternate FDA-approved assay platform revealed a TSH concentration of 6.81 µIU/mL, which was clinically concordant. Extensive analytical validation, including serial dilutions, interference checks, and assessment for prozone effect and heterophilic antibodies, failed to identify technical anomalies. Cross-platform evaluation using CLIA and ECLIA-based assays confirmed the discordance as assay-specific. To further investigate potential causes, the patient underwent thorough clinical re-evaluation by the treating physician, including a detailed review of medication history, which revealed no use of exogenous thyroid hormones or drugs known to interfere with TSH assays. Genomic DNA was subsequently extracted, and the full coding region of the TSHβ subunit gene was sequenced. Bioinformatic analysis using NCBI reference databases did not identify any pathogenic variants, suggesting the possibility of an assay-specific epitope alteration or a structurally atypical TSH isoform contributing to the immunoassay interference [4].

TSH estimation was performed using the Siemens Atellica IM module (Siemens Healthineers, USA) with Siemens reagents at our lab. For comparative analysis, the Alinity i analyzer (Abbott, USA) and Roche Cobas, Switzerland was also used with corresponding reagents. A significant discrepancy was observed in TSH values below 0.008 µIU/mL on the Siemens Atellica platform, which showed poor clinical correlation and lacked comparability with the Alinity i results.

CLIA (Siemens Atellica IM TSH3-UL): Third-generation chemiluminescent immunoassay employing:
•Anti-FITC monoclonal antibody covalently bound to paramagnetic particles
•FITC-labeled anti-TSH capture mouse monoclonal antibody
• Tracer with proprietary acridinium ester and anti-TSH mouse monoclonal antibody conjugated to BSA
• Direct relationship between TSH concentration and relative light units (RLUs)

ECLIA (Roche Cobas): Electrochemiluminescent sandwich immunoassay (18-minute duration):
• 1st incubation: 50 µL sample with biotinylated monoclonal TSH-specific antibody and ruthenium complex-labeled antibody
• 2nd incubation: Streptavidin-coated microparticles for solid-phase binding
• Detection via chemiluminescent emission after voltage application

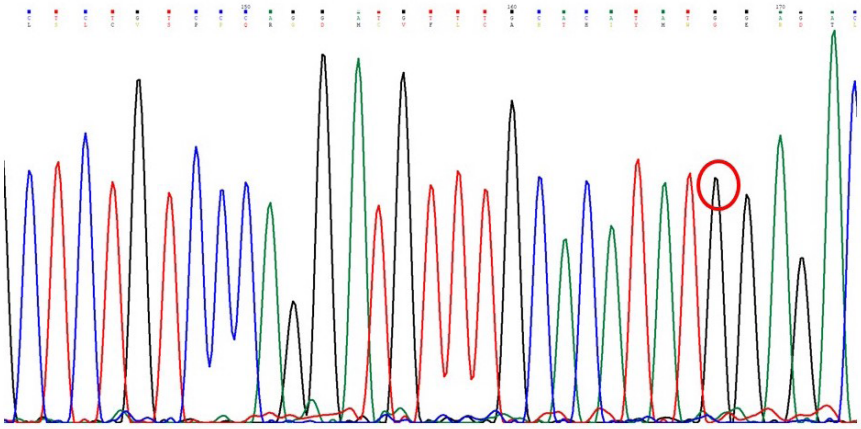
Table 1: Consistent pattern demonstrates reproducible platform-specific interference affecting CLIA methodology while ECLIA maintains detection capability for the variant TSH, confirming the genetic variant's differential impact on antibody recognition systems.

Testing Instances	Siemens Atellica (CLIA)	Roche Cobas (ECLIA)
1st Testing	<0.008 µIU/mL	6.81 µIU/mL
2nd Testing	<0.008 µIU/mL	10.3 µIU/mL
3rd Testing	<0.008 µIU/mL	4.1 µIU/mL

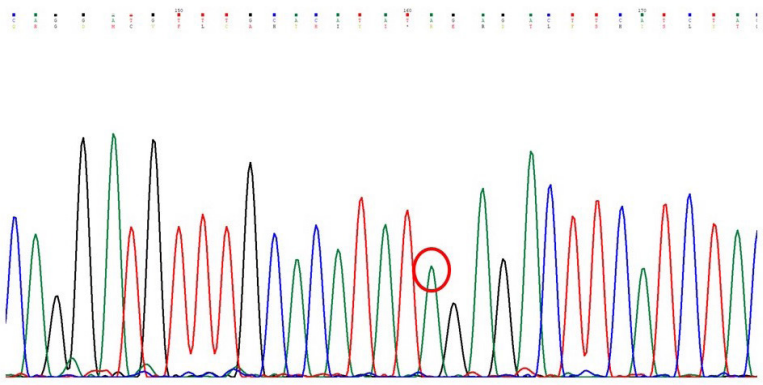
To investigate the cause of this discrepancy, Sanger sequencing of the entire coding region of the TSHB gene was performed. For variant detection, DNA was isolated using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) with automated extraction performed on the QIACUBE system (QIAGEN, Germany). Primers targeting exon 2 and exon 3 of the TSHβ gene were designed using the NCBI Primer Designing Tool and synthesized by Eurofins Genomics India Pvt. Ltd. (Bangalore, India). PCR amplification was conducted using the Bio-Rad C1000 Touch Thermal Cycler (California, USA), with the reaction mixture prepared using Takara PCR Master Mix (Clontech / DSS-Takara, New Delhi, India). The PCR products were purified using Exo-SAP IT (Thermo Fisher Scientific, USA). Sequencing was carried out using Invitrogen Sequencing Master Mix (India) on an ABI 3500 Genetic Analyzer (USA). PCR conditions were as follows: initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 56.3°C for exon 2 and 60°C for exon 3 for 45 seconds, and extension at 72°C for 45 seconds; with a final extension at 72°C for 5 minutes. The PCR reaction mix (total volume 25 µL) consisted of: 14.3 µL distilled water, 2.5 µL of 10× PCR buffer, 1.0 µL of 20.5 mM dNTPs, 1.0 µL each of 10 pmol/µL forward and reverse primers, 5.0 µL of DNA template (20 ng/µL), and 0.2 µL of Taq polymerase (5 U/µL).

This revealed a homozygous A-to-G nucleotide substitution in exon 3, resulting in an arginine-to-glycine amino acid change at codon 75 (R75G) fig. (A) and (B) in the mature TSH β-subunit protein (RefSeq: NP_000540.2). No such variant was detected in a control individual with normal TSH levels, supporting the hypothesis that the R75G mutation interferes with antibody recognition in our assay, rather than reflecting a true deficiency of TSH.

Figure 1: Sanger sequencing chromatograms of the TSHB gene. (A) Patient sample showing a homozygous c.223A>G (R75G) variant in exon 3 (red circle). (B) Control sample with wild-type sequence at the same position.



Case(A)



Control(B)

The alignment shows a homozygous A>G substitution at nucleotide position c.223, resulting in an arginine-to-glycine substitution at codon 75 (R75G), indicated by a red box.

Figure 2: Alignment of the patient's TSHB gene sequence with the reference sequence (NP_000540.2).

Range 3: 505 to 624 Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
217 bits(117)	2e-60	119/120(99%)	0/120(0%)	Plus/Plus

```
Query  150  CAGGATGTTTGCACATATGGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCA  209
            ||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  505  CAGGATGTTTGCACATATAGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCA  564

Query  210  CTCCATGTTGCTCCCTATTTTTCCTATCCTGTTGCTTTAAGCTGTAAGTGTGGCAAGTGC  269
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  565  CTCCATGTTGCTCCCTATTTTTCCTATCCTGTTGCTTTAAGCTGTAAGTGTGGCAAGTGC  624
```

Range 4: 402 to 505 Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
187 bits(101)	1e-51	103/104(99%)	0/104(0%)	Plus/Plus

```
Query  107  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCCCAGGATGTTTGCACATA  166
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  402  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCCCAGGATGTTTGCACATA  461

Query  167  TGGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCAC  210
            | ||||||||||||||||||||||||||||||||||||||||||
Sbjct  462  TAGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCAC  505
```

Range 5: 342 to 384 Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
80.5 bits(43)	2e-19	43/43(100%)	0/43(0%)	Plus/Plus

```
Query  107  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCC  149
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  342  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCC  384
```

Discussion

Immunoassay-based measurement of thyroid-stimulating hormone (TSH) is a cornerstone of thyroid function testing. However, in rare cases, discordant TSH results may arise due to interference caused by structural variants of the TSH molecule. We report a case in which an undetectable TSH concentration was observed using a routine immunoassay platform, while clinical evaluation and free thyroid hormone levels indicated a euthyroid state. Subsequent genetic analysis identified a homozygous missense mutation in exon 3 of the TSHB gene, resulting in an arginine-to-glycine substitution at codon 75 (R75G) of the mature TSH β-subunit protein. This mutation likely alters the conformation of the TSH molecule in a manner that impairs recognition by specific antibodies used in certain immunoassays.

The identified R75G mutation has been previously described by D Shaki et al. in individuals of Ashkenazi Jewish ancestry, where it was similarly associated with anomalously low or undetectable TSH concentrations despite preserved thyroid function. This mutation is postulated to affect a key epitope targeted by monoclonal antibodies in specific TSH immunoassays. Importantly, the affected patients in those studies also displayed normal levels of free thyroxine (FT4) and triiodothyronine (FT3), as in our case, supporting the notion that the mutation compromises immunoreactivity rather than biological activity of the hormone [5].

To validate the hypothesis of assay interference, we analyzed the same patient sample using two additional TSH immunoassay platforms. Both alternative assays yielded measurable TSH concentrations, in stark contrast to the undetectable result on our primary system. This inter-platform discrepancy reinforces the role of the R75G variant in altering the immunoreactive profile of TSH, likely through conformational changes that mask or disrupt epitope-antibody interactions specific to certain assay designs.

While this particular mutation was consistent with the findings of Shaki et al., similar phenomena have been described in the literature involving other TSH variants. For example, J Drees et al. reported a distinct TSH β-subunit variant causing falsely low TSH values due to immunoassay interference. Although the molecular basis differed, the clinical presentation and diagnostic challenge were similar, highlighting a broader issue of variant-dependent immunoassay limitations [6].

Our findings underscore a critical limitation of various current TSH immunoassays: their vulnerability to genetic variation in the hormone structure, particularly when such variants occur within or near epitopes used for antibody binding. These cases demonstrate the necessity of considering genetic causes when TSH levels are unexpectedly low and discordant with the clinical picture. In such situations, confirmation with alternative immunoassay platforms or functional bioassays may be warranted.

Furthermore, this case exemplifies the importance of assay transparency and epitope mapping in test development, especially in diverse populations where rare variants such as R75G may be under recognized. Broader awareness of such mutations and their analytical consequences will improve diagnostic accuracy and prevent misclassification of thyroid status, especially in asymptomatic individuals or during routine screening. Literature reports a higher prevalence of certain genetic variants in Asian populations, including Indian Jews, with our index case from eastern India aligning with this geographic pattern. Current evidence suggests that ethnic-specific genetic architectures contribute to the greater prevalence observed in Asian populations compared to European and North American groups. Clinically, this underscores the need for stringent monitoring when TSH values are discrepant without clear clinical correlation, alongside the use of alternative testing platforms as the best current practice. Patient education about genetic variations and genetic counseling are also essential to support informed healthcare decisions. Moving forward, prospective studies are necessary to better define the prevalence of these variants within different ethnic groups and to develop diagnostic strategies tailored to specific populations [5, 6].

Conclusion

This case highlights the clinical and analytical implications of a homozygous R75G mutation in the TSHB gene, which leads to falsely low or undetectable TSH levels on specific immunoassay platforms due to epitope interference. The mutation does not impair TSH biological activity but disrupts antibody recognition, resulting in assay-specific underestimation. The patient could have modification like glycosylation in TSH due to the variant in TSHβ subunit gene, may have altered TSH epitope expression which induced variable antibody recognition which may be the cause for discordant TSH levels in our laboratory reports. Recognition of such variant-induced assay interference is essential to avoid misdiagnosis and inappropriate clinical management. Comparative testing across multiple assay platforms and genetic analysis should be considered when TSH results are discordant with clinical findings. Standardization and Harmonization of TSH assay may limit such discrepancies in the results.

Future Research Looking ahead, we aim to collaborate with genetic research teams to initiate a longitudinal, multicentric prospective study, contingent on funding and ethical approvals. This research will focus on characterizing ethnic-specific variant prevalence and analyzing platform-specific interference patterns. The ultimate goal is to develop harmonized detection strategies that enhance diagnostic accuracy across diverse populations.

Conflict of interest

Authors do not have any conflict of interest.

Artificial intelligence statement

The authors used Grammarly and ChatGPT to help make the writing clearer.

Disclosure

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgment: During the preparation of this manuscript, the authors Grammarly, OpenAI's language(ChatGPT) model to improve clarity and coherence. The authors reviewed and edited the final content and take full responsibility for its accuracy.

Ethical Approval

This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. The study is approved from Institute Ethical Committee TMC-IEC III, Tata Memorial centre Advanced Centre for Treatment Research & Education in Cancer, Kharghar, Navi Mumbai -410210, India vide approval letter Rr. No.524 dated 09 July 2025.

Informed consent statement

Informed written consent was obtained from the patient.

Funding
No funding was obtained for this research.

Availability of data and materials
Data will be provided on request.

Consent for publication
All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Author Contributions
Avinash Pagdhune contributed to conceptualization, original draft preparation, formal analysis, review and editing, and project administration. Poonam Gera was involved in Conceptualization, visualization, methodology, validation, and formal analysis. Preeti Chavan contributed to resources, methodology, and supervision. Sujeet Kamtalwar participated in resources, investigation, and data curation. Ashwini More provided resources. Varsha Jadhav contributed to methodology, software, Visualization, and validation. Prafulla Parikh was involved in resources and supervision. Sharda Haralkar contributed to methodology. Rajni Mohite and Madhura Patil were involved in methodology. Rushikesh Samal contributed to data curation and resources. Rajiv Sarin was responsible for supervision and resources.

References

1. Pirahanchi Y, Toro F, Jialal I. Physiology, thyroid stimulating hormone. 2023 May 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. PMID: 29763025. (accessed– 13/02/2024)
2. Sterenborg RB, Galesloot TE, Teumer A, Netea-Maier RT, Speed D, Meima ME, Visser WE, Smit JW, Peeters RP, Medici M. The effects of common genetic variation in 96 genes involved in thyroid hormone regulation on TSH and FT4 concentrations. The Journal of Clinical Endocrinology & Metabolism. 2022;107(6):e2276-e2283. doi: 10.1210/clinem/dgac136. PMID: 35262175; PMCID: PMC9315164. (accessed– 13/02/2024)
3. Pappa T, Johannesen J, Scherberg N, Torrent M, Dumitrescu A, Refetoff S. A TSHβ variant with impaired immunoreactivity but intact biological activity and its clinical implications. Thyroid. 2015;25(8):869-876. doi: 10.1089/thy.2015.0096. Epub 2015 Jun 15. PMID: 25950606; PMCID: PMC4533086. (accessed– 13/02/2024)
4. Favresse J, Burlacu MC, Maiter D, Gruson D. Interferences with thyroid function immunoassays: clinical implications and detection algorithm. Endocrine reviews. 2018;39(5):830-850. doi: 10.1210/er.2018-00119. PMID: 29982406. (accessed– 13/02/2024)
5. Shaki D, Eskin-Schwartz M, Hadar N, Bosin E, Carmon L, Refetoff S, Hershkovitz E, Birk OS, Haim A. TSHB R75G is a founder variant and prevalent cause of low or undetectable TSH in Indian Jews. European Thyroid Journal. 2022;11(1). doi: 10.1530/ETJ-21-0072. PMID: 34981755; PMCID: PMC9142811. (accessed– 13/02/2024)
6. Drees JC, Stone JA, Reamer CR, Arboleda VE, Huang K, Hrynkow J, Greene DN, Petrie MS, Hoke C, Lorey TS, Dlott RS. Falsely undetectable TSH in a cohort of South Asian euthyroid patients. The Journal of Clinical Endocrinology & Metabolism. 2014;99(4):1171-1179. doi: 10.1210/jc.2013-2092. Epub 2013 Jan 1. PMID: 24423284. (accessed– 13/02/2024)