



A germline variant of ring finger protein 43 in an early onset, treatment-resistant metastatic gastric cancer: a case report

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Background: Ring finger protein 43 (RNF43) is an E3 ubiquitin-protein ligase that functions as a negative regulator of the Wnt signaling pathway by mediating the ubiquitination, endocytosis, and subsequent degradation of Frizzled receptors within the Wnt receptor complex. It exerts its effects on both canonical and non-canonical Wnt signaling pathways.

Case Description: This case report describes a 49-year-old female patient with a significant family history of cancer and parental consanguinity who was diagnosed with treatment-resistant stage IV gastric adenocarcinoma. Genomic profiling conducted via liquid biopsy identified a missense variant in RNF43 exon 9 (NM_017763.6, c.1948C>T; Arg650Ter) with a high variant allele frequency (VAF) of 49.5%. Confirmation of the R650* variant at the germline level underscores its clinical significance in early onset gastric cancer (GC) pathogenesis.

Conclusions: While interpretations of its pathogenicity vary in the ClinVar database, the application of the American College of Medical Genetics (ACMG) criteria suggests its potential involvement in cancer pathogenesis. This report highlights the necessity for further research to elucidate the role and impact of RNF43 in GC progression and develop specific preventive measures for affected families as genetic testing and counseling in high-risk families.

Keywords: Ring finger protein 43 (RNF43); germline variant; early-onset; gastric cancer (GC); case report

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Introduction

Gastric cancer (GC) is a significant public health concern in Chile, with an age-standardized incidence rate of 188.7 per 100,000 inhabitants, positioning it among the three primary causes of cancer-related mortality (Globocan 2022). This malignancy is associated with substantial morbidity due to the symptomatic burden, postoperative complications, and adverse effects of systemic treatments. In the field of precision medicine, the identification of genetic mutations in these neoplasms has emerged as a crucial aspect for improving patient care (1).

Ring finger protein 43 (RNF43), which encodes a transmembrane E3 ubiquitin ligase, has emerged as a novel tumor suppressor and a critical regulator of the Wnt/ β -catenin signaling pathway (2). RNF43 comprises two distinct functional domains: the N-terminal domain, primarily involved in inhibiting Wnt/ β -catenin signaling and featuring a region that interacts with R-spondins (RSPO) or Frizzled receptor (FZD), alongside an intracellular RING domain; and the C-terminal domain, housing the Dishevelled-2 (DVL2) binding region responsible for mediating communication between RNF43 and FZD (3). This protein is postulated to negatively regulate Wnt signaling, resulting in modifications to the subcellular distribution of these receptors, subsequently reducing their levels on the cell surface (4).

Pathogenic somatic and germline variants of RNF43 have

been associated with the development of various cancers, including colorectal, melanoma, renal, hepatocellular, and endometrial cancers, as well as intestinal adenomas (2,4-7).

These findings underscore the significance of RNF43 as a crucial biomarker in GC prognosis, therapeutic interventions, and immunological processes (8-11).

This report presents the case of a 49-year-old woman diagnosed with stage IIIB gastric adenocarcinoma, specifically poorly differentiated signet ring cell carcinoma. The patient was found to harbor a novel germline variant of RNF43, which was hypothesized to be associated with early-onset gastric cancer (EO-GC), metastasis, and treatment resistance. Notably, the patient's family history is significant for cancer, including GC, at an early age, and the patient's parents are consanguineous. This case elucidates the potential implications of RNF43 variants in familial GC and underscores the importance of genetic testing and counseling in high-risk families. We present this article in accordance with the CARE reporting checklist (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2025-77/rc>).

Case presentation

A 49-year-old female patient with a significant family history of diverse malignant neoplasm, including GC in her father and paternal uncle at an early age, her parents were consanguineous. Patient presented with stage IIIB diffuse type gastric adenocarcinoma with signet ring cells, classified as pT4N3aM0. However, she had no significant comorbidities and was treated with four cycles of neoadjuvant Fluorouracil, Leucovorin, Oxaliplatin, and Docetaxel (FLOT), followed by an extended total gastrectomy. Postoperative biopsy confirmed a diffuse type of gastric adenocarcinoma with signet ring cells, negative margins, and positive lymph nodes (11/78), and lymphovascular invasion. Despite adjuvant chemotherapy with FLOT, the disease progressed to stage IV, with metastatic peritoneal dissemination. Subsequent first-line chemotherapy combined with immunotherapy failed to halt disease progression (irinotecan-nivolumab), as evidenced by radiological assessment at three months. The patient's condition deteriorated, resulting in mortality in palliative care.

Genomic analysis did not identify high tumor mutational burden in the blood (bTMB) or high microsatellite instability (MSI-H) markers. The absence of MSI-H suggests a lack of genomic instability (*Table 1*). Genetic evaluation highlighted a potential somatic variant with a

Highlight box

Key findings

- A patient presenting with early-onset gastric cancer (EO-GC) refractory to treatment.

What is known and what is new?

- The incidence of EO-GC has increased in recent years, establishing it as a distinct pathological entity.
- This case report presents a young patient with gastric cancer (GC) carrying an ring finger protein 43 (RNF43) mutation and a significant family history of the disease.
- The patient demonstrated resistance to conventional treatment and immunotherapy.

What is implication, and what should change now?

- The presence of an RNF43 mutation in a young patient suggests a potential role of this gene in GC pathogenesis.
- Given the high consanguinity within the family, additional genetic contributors warrant investigation.
- Molecular studies in conjunction with genetic counseling are essential to elucidate the significance of RNF43 in this cancer type.

Table 1 Biomarker findings in a 49-year-old woman with EO-GC

Characteristic	Variant allele frequency (%)
Blood tumor mutational burden	0 muts/Mb
Microsatellite status	MSI-H not detected
Tumor fraction	Elevated tumor fraction not detected
RNF43	R650* (49.5%)
TP53	p295fs*51 (0.19%)

Genetic evaluation of cancer did not identify key biomarkers such as bTMB, MSI-H, or tumor fraction. However, a genetic variant of RNF43 was detected in exon 9 (NM_017763.6, c.1948C>T; p. Arg650Ter), with a high VAF of 49.5%, suggesting its germline origin. Sanger sequencing confirmed the heterozygous presence of R650* variant at the germline level. Additionally, a TP53 variant was identified, with a VAF of 0.19%, indicating its somatic origin. bTMB, tumor mutational burden in the blood; EO-GC, early-onset gastric cancer; MSI-H, high microsatellite instability; RNF43, ring finger protein 43; TP53, tumor protein 53; VAF, variant allele frequency.

high variant allele frequency (VAF) of 49.5% in the RNF43 gene exon 9 (NM_017763.6, c.1948C>T; p. Arg650Ter, known as R650*). This nonsense mutation, R650*, confirmed as heterozygous at the germline level by Sanger sequencing (*Figure 1*) (12,13), was classified as Pathogenic Very Strong (PVS) level 1 (14). Furthermore, the ClinVar database searches yielded three results regarding this variant: two of uncertain significance, and one suggesting potential pathogenicity. In silico simulations assigned a pathogenicity Metascore of 8, supporting its classification as potentially pathogenic (15). Additionally, a TP53 variant, P295fs*51, was identified with a VAF of 0.19% (*Table 1*). Subsequent Sanger sequencing confirmed the variant in one son and its absence in the younger sibling, thereby establishing germline inheritance (*Figure 2*). A comprehensive family genealogical pedigree suggested the potential presence of a germline variant in the patient's son.

Clinical characteristics, imaging findings, and laboratory test results were obtained from medical records. Circulating tumor DNA (ctDNA) from liquid biopsy specimens was analyzed utilizing next-generation sequencing (NGS) to identify cancer-related genes during disease progression. A family pedigree was constructed to elucidate potential relatives harboring the same genetic variant. All procedures performed in this study followed ethical standards in accordance with institutional and national guidelines, as well as the Declaration of Helsinki and its subsequent

amendments. Despite all efforts, written informed consent for publication could not be obtained from the patient or her relatives.

Discussion

An R650* variant within RNF43 was identified at the germline level in a patient with early onset gastric adenocarcinoma. This variant is characterized as a nonsense mutation in RNF43 that results in loss of function (*Figure 1*). RNF43, along with its homolog ZNRF3, both E3 ubiquitin ligases, negatively regulate the availability of FZD on the cell surface. This modulation is precisely regulated by RSPO ligands and their co-receptors, leucine-rich repeat-containing G-protein-coupled receptor (LGR) 4/5/6. The LGR-RSPO-RNF43/ZNRF3 complex inhibits FZD ubiquitination, thereby increasing sensitivity to Wnt ligands. This phenomenon is of clinical significance, particularly in cancers exhibiting loss-of-function mutations in RNF43 and gain-of-function mutations in RSPO2/3 (16). Novel therapeutic approaches targeting the Wnt pathway, including porcupine inhibitors (PORCN), anti-FZD agents, and anti-RSPO antibodies, have demonstrated promising results in preclinical studies (17-20).

Considerable attention should be directed towards mutations occurring at both the amino and carboxyl termini of the RNF43 protein, as evidence suggests that they may induce distinct biological effects. Nonsense mutations in the gene result in non-functional proteins due to the truncation of the E3 ligase domain. Conversely, certain missense mutations within the RING domain may lead to the loss of Wnt signaling activity (21). This case report describes the identification of a germline RNF43 variant, R650*, in a female patient with refractory EO-GC. The detection of this variant in one of her offspring supported a hereditary pattern. A primary limitation inherent to this case report is that it cannot establish a definitive causal relationship between the identified germline RNF43 R650* variant and the somatic TP53 variant, and the patient's aggressive disease phenotype or treatment resistance. While the findings are suggestive given the biological context, the observed associations must be interpreted with caution. We acknowledge the potential influence of unmeasured confounders, including other genetic or environmental factors, which cannot be fully excluded in a single-patient study. Furthermore, this case presented concurrent mutations involving germline RNF43 and somatic TP53. Furthermore, while a comprehensive gene panel was used,

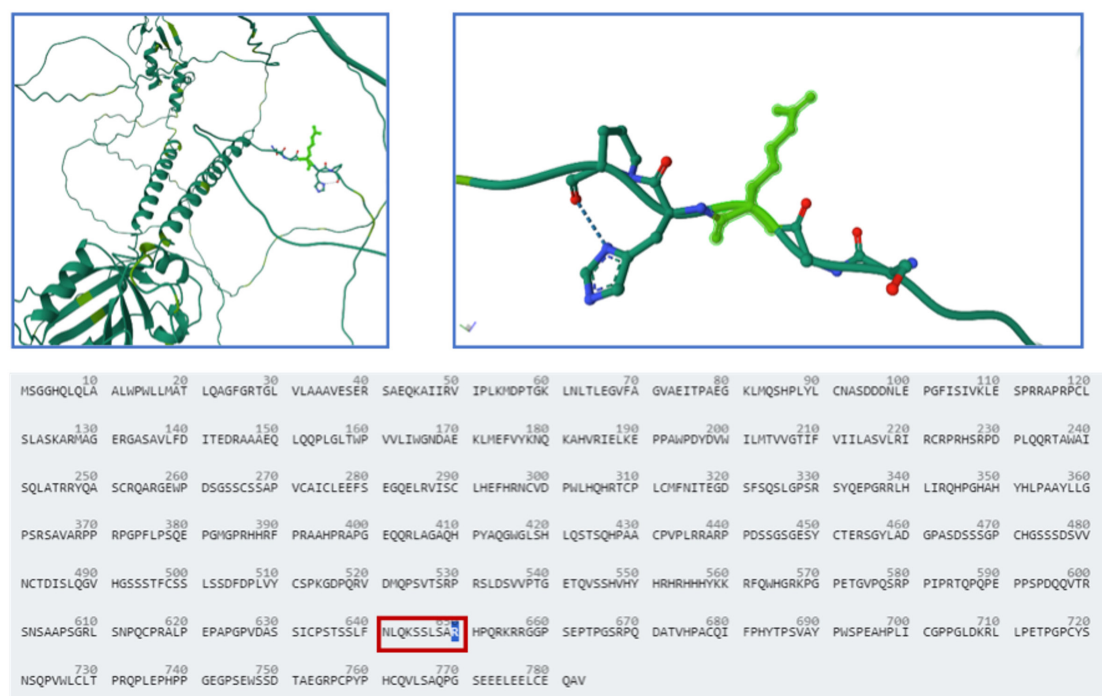


Figure 1 RNF43 R650* variant in early onset gastric cancer. The RNF43 wild-type protein full three-dimensional structure, as predicted by the AlphaFold Protein Structure Database (ebi.ac.uk), is presented. The arginine residue (ARG 650) is depicted in the left panel (green). The RNF43 protein three-dimensional structure (balls and stick model) is illustrated, featuring a magnified view of the ARG 650 residue, shown in the same color (right panel). The RNF43 isoform-1 full amino acid canonical sequence (comprising 783 amino acids) is displayed, with the ARG 650 residue highlighted (red box) and the location of the stop signal indicated (inferior panel). [Sequence obtained from UniProt, RNF43-E3 ubiquitin-protein ligase RNF43-Homo sapiens (Human)|UniProtKB|UniProt] (12,13). RNF43, ring finger protein 43.

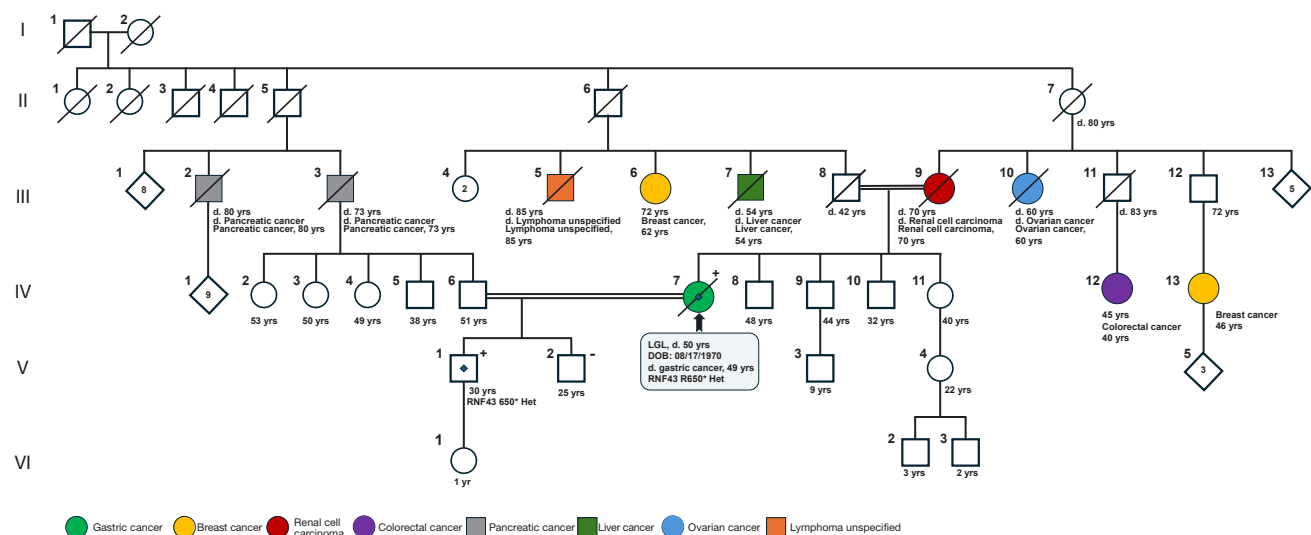


Figure 2 Family pedigree of a 49-year-old woman with an RNF43 germline variant. Pedigree analysis of the family revealed substantial consanguinity and a high prevalence of cancer among family members. Genetic analysis identified the same RNF43 germline variant (sig+) in one offspring, indicating its transmission within the family lineage. DOB, date of birth; LGL, living generation label; RNF43, ring finger protein 43.

the potential contribution of variants in genes not included in the panel or non-coding regions cannot be excluded. Pathogenicity assessment of the RNF43 R650* variant relied on sequence interpretation guidelines, database information, and *in silico* tools, as direct functional assays were not performed. The hypothesized functional interplay between the RNF43 and TP53 variants is extrapolated from prior *in vitro* research and requires specific experimental validation. Previous *in vitro* studies have demonstrated that RNF43 interacts with TP53 and suppresses its transcriptional activity. This interaction, mediated by NEDD4-like ubiquitin-protein ligase-1 (NEDL1), may contribute to carcinogenesis. Additionally, RNF43 inhibited TP53-mediated transcription and attenuated apoptosis induced by ultraviolet irradiation *in vitro*. These findings suggest that RNF43 is implicated in TP53- and NEDL1-mediated apoptotic pathways, potentially contributing to the resistance mechanisms associated with colorectal carcinogenesis (22). It is hypothesized that the simultaneous occurrence of both mutated genes in this patient may have contributed to treatment resistance. The clinical interpretation of the RNF43 R650* variant is complicated by conflicting entries in public databases such as ClinVar, underscoring the difficulties in classifying rare variants with limited functional data. Although the variant meets PVS1 criteria under ACMG/AMP guidelines due to its predicted null effect, which would support the proposed reclassification as potentially pathogenic, further research and case aggregation are necessary to fully elucidate the penetrance and specific risks associated with RNF43 germline variants in GC.

For example, since RNF43 acts as a tumor suppressor, a second hit in the non-mutated gene allele was needed, involving complex chain of events that are not easily identifiable during EO-GC tumorigenesis. Moreover, a IIIB disease stage at the time of diagnosis revealed an advanced and complex clinical scenario. Of note, a survival analysis in a Japanese registry of 118,367 patients with primary gastric carcinoma managed by surgical resection, stage IIIB data analyses showed an overall 5-year survival of 34.8% (23).

Regarding the specific RNF43 heterozygous germline genetic variant in this case, a recent study (24), using complex *in vitro* and *in vivo* assessments plus the study of human biopsies, showed, among, other conclusions that loss of RNF43 function conferred resistance to DNA damage-induced cell death in AGS cell lines after γ -radiation, influenced susceptibility to DNA damage-Inducing chemotherapeutics such as 5-fluorouracil (5-FU) or

cisplatin, also in AGD cell lines, and that RNF43 is involved in *H. pylori*-induced DNA damage response using in both *in vitro* and *in vivo* models, the latter using *Rnf43* ^{Δ Ex8} mutant mice with a loss of the RNF43 ring domain by introducing a deletion in the exon 8 by CRISPR/Cas9 technology.

Of note, in the same study, by analyzing human gastric tissue samples, 5 of 15 cases harbored a mutation in TP53 and two diffuse type cases had a RNF43 mutation.

Finally, the observed resistance to standard chemotherapy and immunotherapy is likely multifactorial. While we postulate a potential role for the identified RNF43 and TP53 variants, this case report did not assess other potential resistance mechanisms, such as epigenetic alterations, tumor microenvironment interactions, or pharmacokinetic factors, which warrant further investigation.

Conclusions

The incidence of EO-GC has increased in recent years, establishing it as a distinct pathological entity. We present a case of GC in a young patient harboring mutation in RNF43 and TP53. The patient exhibited a significant family history of cancer, suggesting a potential hereditary component. Following diagnosis, the patient has demonstrated resistance to conventional treatments and immunotherapy.

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None.

Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-2025-77/rc>

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study followed ethical standards in accordance with institutional and national guidelines, as well as the Declaration of Helsinki and its subsequent amendments. Despite all efforts, written informed consent for publication could not be obtained from the patient or her relatives.

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