

Germline Whole-Gene Deletion of SDHA in a Carotid Body Paraganglioma Identified by Whole-Genome Sequencing and Confirmed by MLPA: A Case Report and Review of the Literature

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Case Report

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Abstract

Background: Carotid body paragangliomas are frequently associated with germline variants in succinate dehydrogenase (*SDH*) genes. Among these, *SDHA* variants are particularly challenging to diagnose due to low penetrance and the presence of highly homologous pseudogenes. Standard diagnostic workflows, including whole-exome sequencing and targeted panels, often fail to detect large structural variants, such as whole-gene deletions, leading to potential underdiagnosis. This case is the first to report a germline whole-gene deletion of *SDHA* identified via whole-genome sequencing (WGS), providing definitive physical evidence of the Knudson two-hit hypothesis through the somatic loss of the remaining allele in the tumor tissue.

Case presentation: A 63-year-old female presented with a 2-cm, left carotid body tumor. There was no relevant family history. The tumor was successfully resected, and histopathology confirmed a paraganglioma. While immunohistochemistry showed a loss of both *SDHA* and *SDHB* expression, WGS of peripheral blood revealed what is, to our knowledge, the first reported germline whole-gene deletion of the *SDHA* gene (copy number ≈ 1.1) in the region chr5:210,651–314,171. Notably, this type of large structural variant is potentially elusive to standard genetic analysis workflows. Subsequent WGS of the tumor tissue confirmed biallelic inactivation through the loss of the remaining allele (copy number ≈ 0.86), representing a classic two-hit mechanism. This large-scale deletion of all *SDHA* exons was further validated using multiplex ligation-dependent probe amplification (MLPA), confirming it as the pathogenic driver. The patient remains under rigorous long-term follow-up given the reported 15% risk of malignancy associated with *SDHA* variants.

Conclusions: This case highlights the clinical utility of WGS in overcoming the inherent limitations of exome-based sequencing for *SDHA* analysis. When clinical suspicion remains high - specifically in cases with *SDHA*-negative immunohistochemistry but negative standard genetic testing - WGS or MLPA should be employed to detect hidden structural variants. Accurate molecular diagnosis is essential not only for understanding the mechanism of tumorigenesis but also for tailoring oncological surveillance and genetic counseling for the patient and their family.

Background

Carotid body paragangliomas are rare, highly vascularized parasympathetic paragangliomas arising from the carotid body at the carotid bifurcation, accounting for less than 0.5% of all head and neck tumors (1–3). Approximately 36–52% of carotid body paragangliomas are driven by germline variants in succinate dehydrogenase (*SDH*) genes. The *SDH* complex consists of four subunits (*SDHA*, *SDHB*, *SDHC*, and *SDHD*) and its assembly factor (*SDHAF2*). Historically, *SDHB* and *SDHD* have been identified as the most frequent causative genes in genetic studies utilizing whole-exome sequencing (WES) or targeted next-generation sequencing (NGS) panels. In contrast, *SDHA* variants have been reported as relatively rare, accounting for only 2–10% of cases (4, 5).

However, this perceived rarity may be partially attributed to the significant diagnostic challenges inherent in the *SDHA* locus. While the penetrance for *SDHB* and *SDHD* variants is reported to be 44% and 90% by age 70, respectively, the penetrance for *SDHA* variants is notably lower at approximately 10% by the same age. Consequently, *SDHA*-related cases frequently lack a positive family history, which is a characteristic feature of this genotype(6–8). More importantly, the presence of multiple highly homologous pseudogenes frequently interferes with the accuracy of short-read sequencing, and standard WES or NGS panel workflows are notoriously limited in their ability to detect large structural variants(SVs) or whole-gene deletions(9). Consequently, pathogenic *SDHA* alterations may remain "hidden" or be missed by conventional diagnostic pipelines.

The identification of these variants is clinically crucial, as *SDHA* mutations are associated with a reported 15% risk of malignancy and distant metastasis(10–14). According to the Knudson two-hit hypothesis, *SDHA* functions as a tumor suppressor where biallelic inactivation is required for tumorigenesis(15). While immunohistochemistry (IHC) for *SDHA*/*SDHB* serves as an effective screening tool to predict protein loss(4, 9, 15, 16), whole-genome sequencing (WGS) provides superior diagnostic yield by identifying large-scale genomic alterations that escape detection by standard methods.

To our knowledge, this report describes the first case of a carotid body paraganglioma caused by a germline whole-gene deletion of the *SDHA* gene identified via WGS. We provide definitive evidence of biallelic inactivation through the subsequent loss of the remaining allele in the tumor tissue, highlighting the utility of WGS in cases where standard workflows fail to identify the underlying genetic driver.

Case Presentation

Clinical History

A 63-year-old Japanese female presented to our department with an asymptomatic, slow-growing mass in the left side of her neck. She had a medical history of appendicitis, mastopathy, bronchial asthma, and cholelithiasis. Physical examination revealed a firm mass with decreased mobility at the level of the left carotid bifurcation. Importantly, there was no reported family history of paraganglioma, pheochromocytoma, or other hereditary cancer syndromes, suggesting an apparently sporadic presentation.

Imaging and surgical management

Magnetic resonance imaging (MRI) revealed a 2-cm tumor with flow voids located immediately above the left carotid bifurcation, suspicious for a carotid body paraganglioma (Fig. 1). Angiography and a Matas test confirmed sufficient collateral cross-flow.

A 2-cm mass, exhibiting low-to-isointense signal on T1-weighted image (a) and heterogeneous hyperintensity on T2-weighted image (b), is located directly above the left carotid bifurcation, splaying

the internal and external carotid arteries on axial time-of-flight MR angiography (c) and coronal T2-weighted fat-suppressed image (d), highly suggestive of a carotid body paraganglioma.

Five months after the initial visit, tumor resection was performed under general anesthesia. Enlarged lymph nodes in the superficial and deep neck were also resected. Intraoperatively, an adventitial defect was identified at the carotid bifurcation. Following ligation of the external carotid artery, the defect was repaired with an autologous external jugular vein patch. The tumor was diagnosed as Shamblyn type II. The postoperative course was uneventful, with no cranial nerve palsy.

Histopathological and Immunohistochemical Examination

The resected tumor and the enlarged superficial and deep neck lymph nodes were submitted for histopathological evaluation.

The tumor showed nested (Zellballen) and map-like growth of atypical cells with clear-to-eosinophilic cytoplasm and hyperchromatic nuclei. IHC was positive for synaptophysin, chromogranin A (weak), and S100 (in sustentacular cells), confirming a paraganglioma. Critically, IHC for both SDHB and SDHA was negative, strongly suggesting an *SDHA* variant (Fig. 2). No lymph node metastases were found.

(a) Hematoxylin and eosin staining shows a classic "Zellballen" nest-like growth pattern of tumor cells with abundant clear-to-eosinophilic cytoplasm. (b–f) IHC analysis: The tumor cells show (b) strong positivity for synaptophysin, and (c) weak positivity for chromogranin A, confirming the diagnosis of paraganglioma. (d) S100 staining highlights the sustentacular cells surrounding the tumor nests. Notably, IHC for both (e) SDHB and (f) SDHA is completely negative in the tumor cells, strongly suggesting an underlying *SDHA* variant. (Scale bars: 100µm in all panels).

Genetic analysis

To identify the genetic driver, we performed WGS using DNA extracted from both peripheral blood and fresh-frozen tumor tissue. In the germline analysis, standard bioinformatics pipelines for SNVs and small indels yielded no pathogenic variants. However, copy number analysis using WGS data revealed loss (CN $\approx$ 1.1) in the region chr5:210,651–314,171; to our knowledge, this represents a novel whole-gene deletion of the entire *SDHA* gene.

Furthermore, WGS analysis of the tumor DNA demonstrated a significant reduction in the *SDHA* locus signal (CN = 0.86), indicating somatic loss of the remaining wild-type allele, consistent with biallelic inactivation (Fig. 3).

Log2 ratio plots of the tumor and germline (blood) samples are shown. The germline sample exhibits a copy number loss (CN $\approx$ 1.1) involving the entire *SDHA* gene region on chromosome 5 (chr5:210,651–314,171). The tumor sample shows a further decrease in copy number (raw CN = 1.59 for the surrounding region and CN = 0.86 for the *SDHA* locus), indicating germline whole-gene deletion with somatic loss of the remaining allele, consistent with biallelic inactivation.

To provide independent validation of this large-scale structural variant, we performed multiplex ligation-dependent probe amplification (MLPA) using the SALSA® MLPA® Probemix P429 SDHA-MAX-TMEM127 kit. The MLPA results confirmed a CN ratio of 0.5 across all exons of the *SDHA* gene in the germline, consistent with the WGS findings (Fig. 4).

The MLPA results confirm a copy number ratio of approximately 0.5 across all exons of the *SDHA* gene. This finding validates the presence of a germline heterozygous deletion of the entire *SDHA* gene, consistent with the WGS results.

Follow-up and outcomes

Based on the molecular diagnosis of an *SDHA*-related paraganglioma, the patient was informed of the approximately 15% risk of malignancy and the low penetrance of the variant. She is currently undergoing regular clinical surveillance with periodic imaging. Genetic counseling and predictive testing for first-degree relatives are planned.

Discussion and Conclusions

Genetic testing is now strongly recommended for all patients with paragangliomas, including carotid body paragangliomas, to guide clinical management and family screening(17, 18). Among the causative genes, *SDHA* represents a unique diagnostic challenge. Since its first report in 2010 as a tumor suppressor gene(15), its identification has been frequently hindered by its complex genomic structure.

A major diagnostic hurdle in *SDHA* analysis arises from the presence of four highly homologous pseudogenes (*SDHAP1-3* and the *SDHAP1* locus), which share approximately 98% sequence identity with the functional gene(9). In standard NGS panels and WES (19), short-read sequences are often misaligned to these pseudogenes or filtered out during quality control due to multi-mapping issues. Furthermore, our case involved a germline whole-gene deletion, a large-scale SV that is notoriously difficult to detect via WES. Because WES focuses on protein-coding regions and relies on the enrichment of targeted exons, it often lacks the uniform coverage required to accurately identify copy number alterations (CNAs) that span across introns and intergenic regions. In contrast, WGS provides unbiased, continuous coverage across the entire genome, allowing for the detection of SVs that escape detection by conventional exome-based pipelines. Indeed, previously reported *SDHA* alterations in paraganglioma have predominantly consisted of single-nucleotide variants (SNVs), while SVs remain exceptionally rare. While a few small-scale structural variants (SVs) involving *SDHA* have been reported in ClinVar and the literature (Table 1) (20), to our knowledge, a large-scale deletion of the entire *SDHA* gene has not been previously documented in any clinical entity, including head and neck paraganglioma.

Table 1
Summary of representative *SDHA* structural variants reported in ClinVar and literature.

Variant (NM_004168.4)	Variant Type	Clinical Significance	Phenotype	Data Source/ Reference
c.897_1260 + 1del	Deletion	Pathogenic	PGL/PCC syndrome 5	Dubard Gault M et al. (2018) (20)
c.769_770 + 77del	Deletion	Likely pathogenic	PGL/PCC syndrome 5	ClinVar(No published report)
c.1001_1064 + 5dup	Duplication	Likely pathogenic	Hereditary cancer syndrome	ClinVar(No published report)
c.1552 – 173_1585dup	Duplication	Uncertain significance	Mitochondrial complex II deficiency	ClinVar(No published report)
chr5:210,651– 314,171	Whole-gene Deletion	Pathogenic	Head and neck paraganglioma	Present case

PGL/PCC: Paraganglioma/Pheochromocytoma.

Beyond identifying the variant, our WGS findings provide definitive physical evidence of the Knudson two-hit hypothesis for *SDHA*-driven tumorigenesis. WGS of the peripheral blood demonstrated a germline "first hit" consisting of a broad loss (CN $\approx$ 1.1) encompassing the entire *SDHA* locus. Crucially, the tumor DNA analysis showed a further reduction in copy number (CN = 0.86), representing the somatic "second hit" via loss of heterozygosity (LOH). While IHC analysis for *SDHA* and *SDHB* showed negative results, which reliably indicated protein loss, it could not distinguish between specific types of underlying mutations. The integration of WGS and MLPA validation allowed us to characterize this biallelic loss as a whole-gene deletion, a novel pathogenic variant that has not been previously reported.

The clinical implications of identifying such "hidden" *SDHA* variants are profound, given the specific risk profile associated with this gene. *SDHA* has a remarkably low penetrance of approximately 10% by age 70, which explains the lack of family history in our patient and many others(6–8). However, the risk of malignancy and distant metastasis in *SDHA*-related cases is reported to be approximately 15%. While 80% of such metastatic cases involve thoracic or abdominal paragangliomas, metastatic disease has also been reported in HNPGLs, though its exact frequency in HNPGLs remains undefined (10–14). Identifying the specific *SDHA* subunit variant is therefore essential for establishing a rigorous long-term surveillance strategy that accounts for this potential risk, particularly since metastatic disease has been reported in head and neck paragangliomas.

This case also has important implications for family management. Although the patient had no apparent family history suggestive of hereditary paraganglioma, the identification of a germline whole-gene deletion of *SDHA* indicates a heritable predisposition and supports referral for genetic counseling. First-degree relatives should be informed of the possibility of carrying the same alteration and offered cascade genetic testing after appropriate counseling. Because *SDHA*-associated disease may show

reduced penetrance(6–8), the absence of a striking family history does not exclude a hereditary background. Recognition of this structural germline alteration is therefore relevant not only for establishing the molecular diagnosis in the index case but also for risk assessment and surveillance planning in the family.

In conclusion, this case demonstrates that in the presence of SDHA-negative IHC, a "negative" result from a standard NGS panel or WES should be interpreted with caution. WGS and MLPA should be considered as secondary diagnostic tiers to uncover large-scale structural alterations that standard workflows may miss. Achieving a precise genetic diagnosis is critical for tailoring lifelong patient management and identifying at-risk family members who may otherwise be overlooked due to the gene's low penetrance.

Abbreviations

CNA Copy number alteration

HNPGL Head and neck paraganglioma

IHC Immunohistochemistry

MLPA Multiplex ligation-dependent probe amplification

MRI Magnetic resonance imaging

NGS Next-generation sequencing

PCC Pheochromocytoma

PGL Paraganglioma

SDH Succinate dehydrogenase

SNV Single-nucleotide variant

SV Structural variant

WES Whole-exome sequencing

WGS Whole-genome sequencing

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Keio University Hospital (Approval Number: 20211069, 20221008). Written informed consent was obtained from the participant. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

H. Oz. served as the principal investigator of this study, and TU served as the principal investigator of the collaborative research institution. IF was responsible for project management, including the development of the overall study protocol and coordination with AMED and participating institutions. SK, H. Oz., and KN conceived and designed the study. SK, H. Oz., and KM collected the patients' data. KM was involved in clinical management, including angiography and perioperative support. H. Oz. and H. Ob. performed the surgical procedure, including the adventitial repair, and conducted postoperative follow-up. AU conducted the immunohistochemical analysis. KN performed the genetic analysis, including whole-genome sequencing and MLPA. SK, H. Oz., and KN wrote the manuscript. H. Oz., KN, and AU offered professional advice about the entire research. All authors read and approved the final manuscript.

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Figures

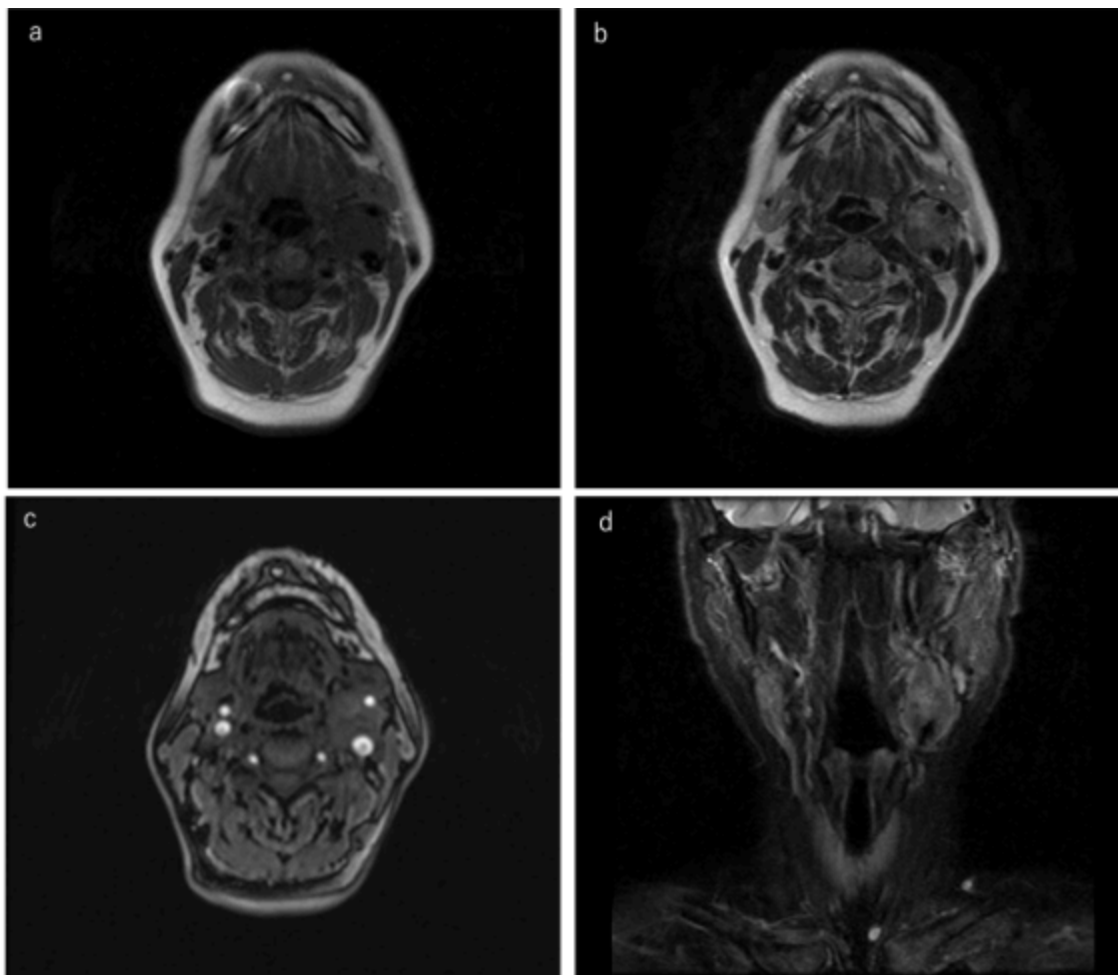


Figure 1

Preoperative magnetic resonance imaging and angiography of the left carotid body tumor.

A 2-cm mass, exhibiting low-to-isointense signal on T1-weighted image (a) and heterogeneous hyperintensity on T2-weighted image (b), is located directly above the left carotid bifurcation, splaying the internal and external carotid arteries on axial time-of-flight MR angiography (c) and coronal T2-weighted fat-suppressed image (d), highly suggestive of a carotid body paraganglioma.

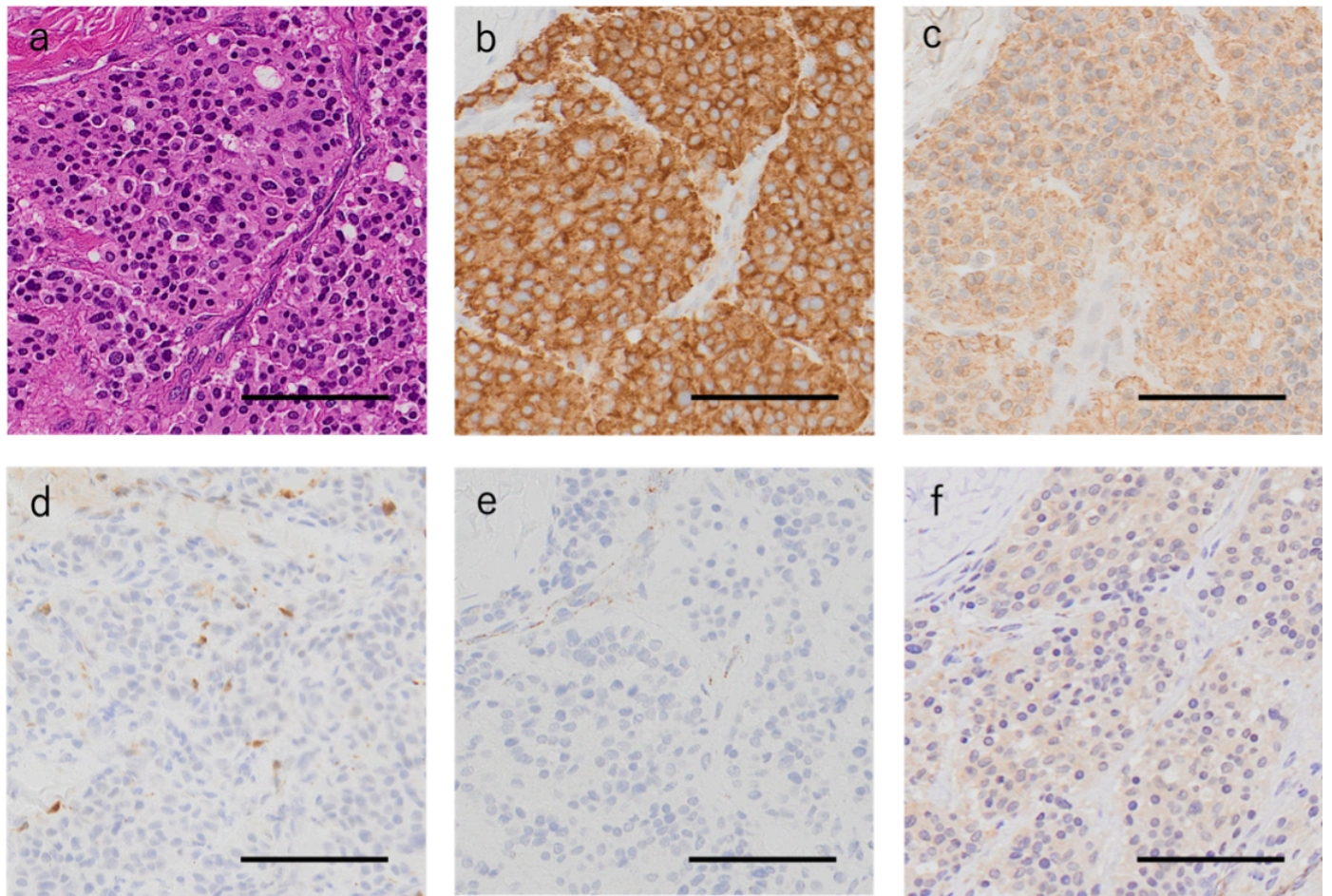


Figure 2

Histopathological and immunohistochemical findings of the resected tumor.

(a) Hematoxylin and eosin staining shows a classic "Zellballen" nest-like growth pattern of tumor cells with abundant clear-to-eosinophilic cytoplasm. (b–f) IHC analysis: The tumor cells show (b) strong positivity for synaptophysin, and (c) weak positivity for chromogranin A, confirming the diagnosis of paraganglioma. (d) S100 staining highlights the sustentacular cells surrounding the tumor nests. Notably, IHC for both (e) SDHB and (f) SDHA is completely negative in the tumor cells, strongly suggesting an underlying *SDHA* variant. (Scale bars: 100 μm in all panels).

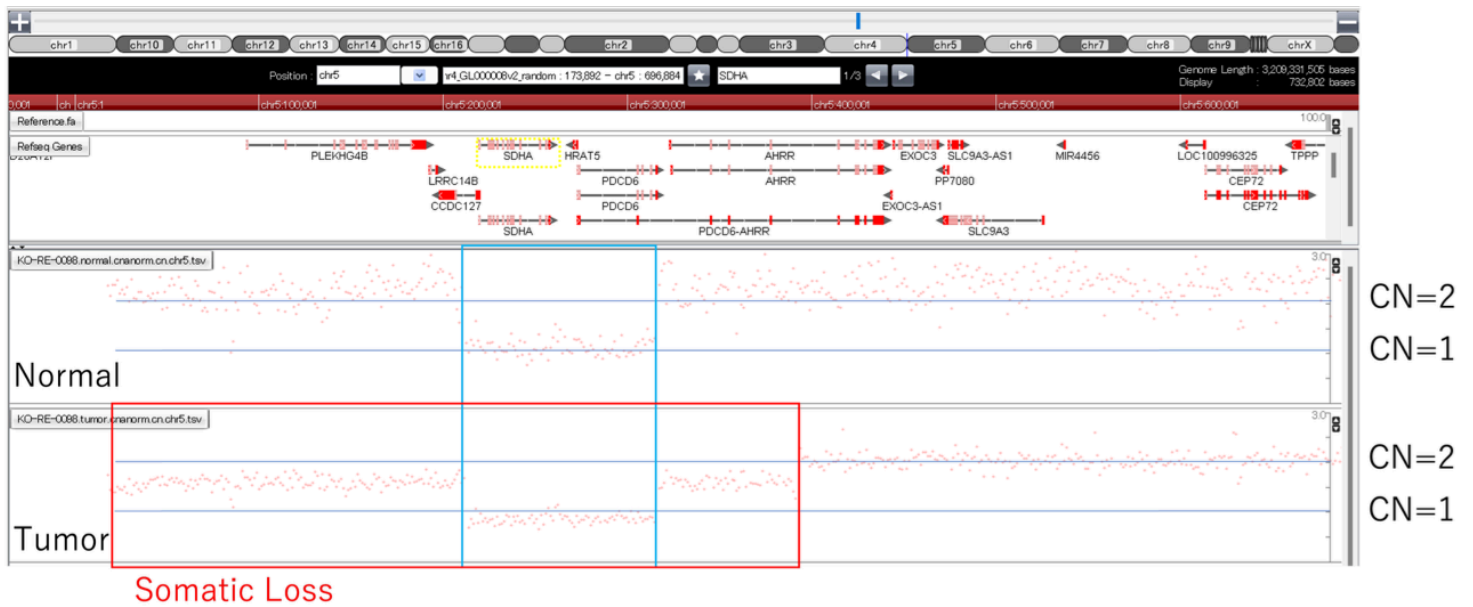


Figure 3

WGS analysis of the *SDHA* locus.

Log2 ratio plots of the tumor and germline (blood) samples are shown. The germline sample exhibits a copy number loss (CN ≈ 1.1) involving the entire *SDHA* gene region on chromosome 5 (chr5:210,651–314,171). The tumor sample shows a further decrease in copy number (raw CN = 1.59 for the surrounding region and CN = 0.86 for the *SDHA* locus), indicating germline whole-gene deletion with somatic loss of the remaining allele, consistent with biallelic inactivation.

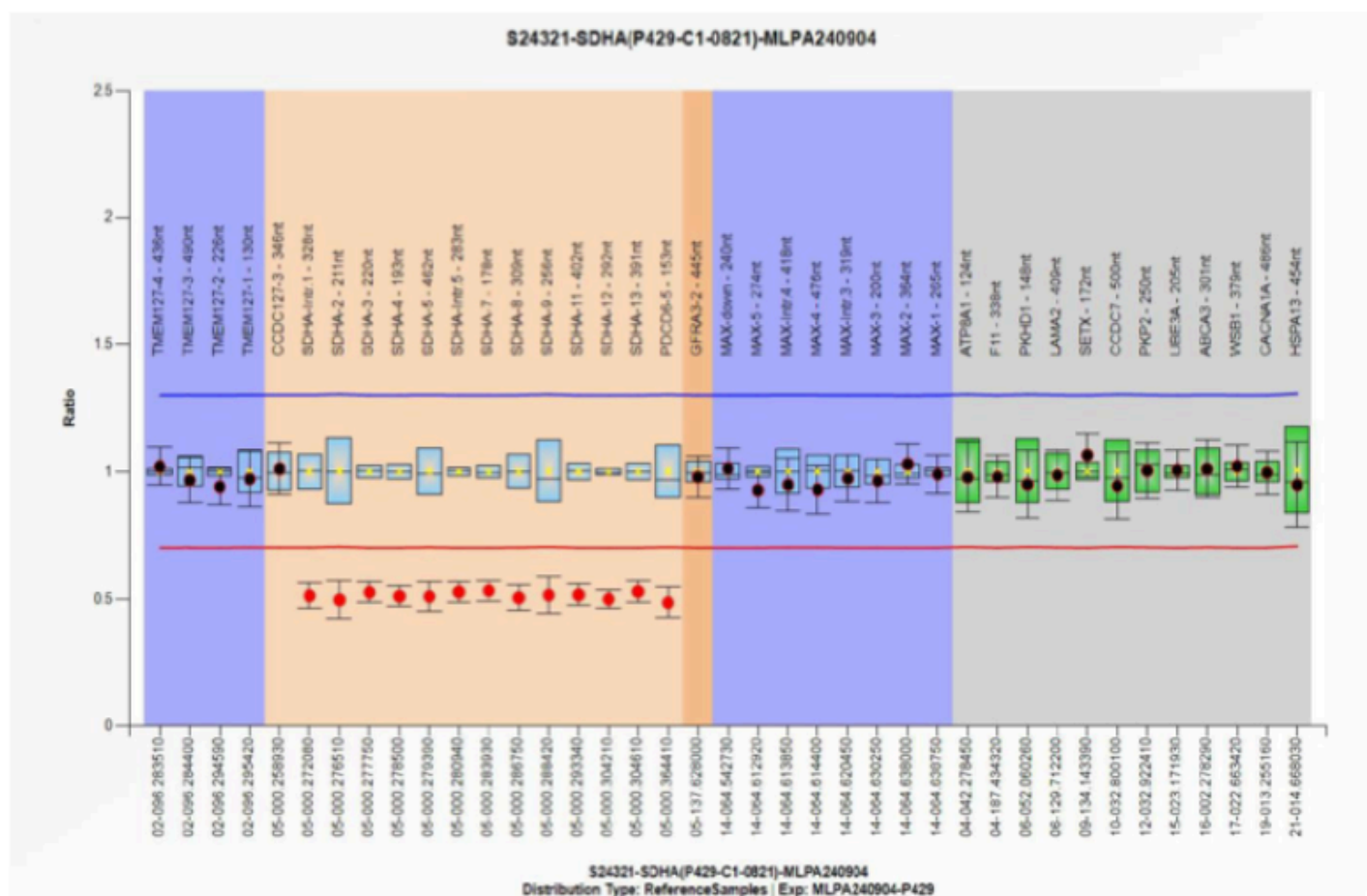


Figure 4

MLPA analysis.

The MLPA results confirm a copy number ratio of approximately 0.5 across all exons of the *SDHA* gene. This finding validates the presence of a germline heterozygous deletion of the entire *SDHA* gene, consistent with the WGS results.