

# Molecular Overview of Major Salivary Gland Carcinomas in Chilean Patients: 2 case reports

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## Case Report

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# Abstract

**Background** Salivary gland tumors (SGTs) are rare neoplasms that account for 8.5 % of head and neck cancers and comprise a highly heterogeneous group of benign and malignant subtypes, according to the World Health Organization. In Chile, epidemiological and molecular data are limited, and comprehensive genomic profiling has rarely been reported. Within our institutional head and neck cancer registry, two patients with malignant major salivary gland carcinomas were identified, each exhibiting distinct molecular profile.

**Case presentation** The first case demonstrated that genomic analysis identified two gene fusions. *BRAF*-driven profile characterized by a dominant *BRAF*(p.V600E), including *TRIM24–BRAF* and *SDHC–RPA1* rearrangements. These findings suggest that MAPK pathway activation, mitochondrial dysfunction, and replication stress occur within a cooperative evolutionary framework. Genomic profiling of the second patient revealed three fusion events, one of which was a fusion-driven tumor dominated by *FGFR2–ETV6* rearrangement. A secondary fusion, *AFG1L–ATF6B* was identified, accompanied by *TP53* alterations and a third intergenic *FGFR2* rearrangement. This profile indicates structural complexity and a strong dependency on *FGFR2* signaling, with additional secondary alterations affecting genes involved in growth signaling and in epigenetic regulation.

**Conclusions** Integrated clinical-molecular analysis revealed a hierarchical organization with dominant driver events and multiple cooperative alterations. These findings highlight the molecular diversity of SGTs and support genomic profiling to identify actionable targets, particularly *FGFR2*, enabling precision oncology approaches in Chilean patients.

## Introduction

Salivary gland tumors (SGTs) are rare malignancies with an estimated incidence of approximately 2.5–3.0 cases/100,000 persons/per year in Western countries and constitute 8.5% of head and neck malignancies, they are characterized by considerable aggressiveness and mortality [1, 2]. According to the World Health Organization (WHO) classification, SGTs comprise a highly heterogeneous group of histological and molecular subtypes with variable biological behaviors [3]. Despite their relative rarity, advanced or recurrent diseases are associated with limited therapeutic options and unpredictable clinical outcomes. Recent advances in next-generation sequencing (NGS) have demonstrated that many SGTs are driven by recurrent gene fusions and pathway-specific oncogenic alterations, including rearrangements involving *BRAF*, *FGFR*, *NTRK*, and *ETV6*, highlighting the clinical relevance of molecular profiling and targeted therapy [4–6].

However, most genomic data originate from North American, European, and Asian populations, whereas molecular epidemiological information from Latin America (LATAM) remains scarce. Population-based studies illustrate geographic variability; for example, in Germany, the age-standardized incidence rate for salivary gland carcinomas is approximately 0.65 per 100,000 persons, with the parotid gland accounting for nearly 78% of the cases [7].

In Chile, salivary gland carcinomas represent less than 5% of head and neck cancers, with an estimated incidence of 0.5 -1.0 cases per 100,000 habitants [8, 9]. Approximately 15.7% of salivary gland tumors are malignant, while 84.3% are benign, and the parotid gland is the most frequently affected site (75–85%). Importantly, molecular characterization is not routinely performed in clinical practice [10, 11].

This lack of local genomic data limits the identification of actionable alterations and the implementation of precision oncology strategies in this population. Regional characterization is essential given the potential geographic variability in the molecular landscapes of tumors. In this context, we report the genomic profiling of two Chilean patients with malignant major salivary gland carcinomas (MSGC), revealing distinct fusion-driven molecular architectures with potential therapeutic implications for precision medicine and future studies.

## Results

### Case Presentation

Two cases of MSGC were retrospectively identified from our institutional database. Molecular analyses were performed using comprehensive genomic profiling, revealing distinct molecular architectures characterized by gene fusions, driver mutations, and hierarchical clonal organization of genomic alterations.

#### Patient 1: BRAF-driven molecular architecture in MSGC

Genomic analysis identified two fusions in this patient, first a dominant *BRAF* (p.V600E) mutation with a variant allele frequency (VAF) of 5.1%, accompanied by a *TP53* (p.L251F) alteration (VAF 4.8%), consistent with major oncogenic events in this type of tumor. A *TRIM24-BRAF* fusion on chromosome 7 was detected (VAF 1.2%), involving a rearrangement that juxtaposed the *TRIM24* promoter to the *BRAF* coding region (including exon 18). This configuration is predicted to drive constitutive *BRAF* expression and sustained activation of the MAPK (MEK-ERK) signaling pathway, promoting enhanced cell proliferation and survival, and potential resistance to therapy (Fig. 1A).

A second structural rearrangement, *SDHC-RPA1* (VAF 1.1%), involving chromosomes 1 and 17, was identified. This fusion links *SDHC* exons 1–6 to the *RPA1* coding region, suggesting a functional interaction between mitochondrial metabolism and DNA replication/repair pathways in tumorigenesis. The predicted biological consequences include mitochondrial dysfunction, DNA damage accumulation, and increased genomic instability, representing a complementary oncogenic mechanism associated with metabolic-genomic stress in MSGC (Fig. 1B).

Additional low-frequency secondary variants included *BCL2L1* (p.V161I), *DCTN1* (p.L1159S), *DNMT3A*, and *PIK3CA*, each detected at low clonal representation (VAF  $\approx$  1.0%). Integrated clinical-molecular score demonstrated a hierarchical structure, with *BRAF* and *TP53* occupying the highest relevance and biological impact, whereas fusion events and other alterations showed moderate to low cooperative

contributions. Low-frequency alterations in *DNMT3A* and *PIK3CA* (VAF  $\approx$  1.0%) clustered in the low-impact region, suggesting late or cooperative events related to epigenetic regulation and PI3K signaling (Fig. 2). An overview of somatic alterations, organized according to the VAF and integrated clinical-molecular score, confirmed this hierarchical organization. Primary driver events included *BRAF* (VAF 5.1%) and *TP53* (VAF 4.8%) mutations, while the *TRIM24-BRAF fusion* (VAF 1.2%) represented a subclonal structural event potentially contributing to MAPK pathway activation. Additional low-VAF alterations included the *SDHC-RPA1* fusion (VAF 1.1%), *BCL2L1* (VAF 1.1%), *DCTN1* (VAF 1.0%), *DNMT3A* (VAF  $\approx$  1.0%), and *PIK3CA* (VAF  $\approx$  1.0%). Overall, these findings support a model of tumor evolution characterized by dominant driver mutations, accompanied by multiple low-frequency cooperative events. In the hierarchical representation, driver genes are shown as red circles, whereas secondary or cooperative alterations are indicated in blue, illustrating the clonal architecture and molecular heterogeneity of the MSGC. (Supplementary Fig. S1).

## Patient 2: FGFR2-centered oncogenic architecture in MSGC

Genomic profiling of the second patient revealed three fusion events, one of which was a fusion-driven tumor dominated by an *FGFR2-ETV6* rearrangement (VAF 30.2%), consistent with a primary clonal driver event. This fusion preserves the FGFR2 kinase domain and is predicted to induce constitutive receptor activation, resulting in sustained signaling through the RAS-MAPK and PI3K-AKT pathways, thereby promoting tumor cell proliferation and survival in MSGC (Fig. 3A).

A secondary fusion, *AFG1L-ATF6B*, was identified at moderate clonal representation and predicted to affect the endoplasmic reticulum (ER) stress and unfolded protein response (UPR) pathways, potentially contributing to tumor cell adaptation and survival under cellular stress conditions (Fig. 3B). In addition, a third structural alteration involving an intergenic (*OPRK1/LOC100507516*)-*FGFR2* rearrangement was detected (VAF 20.5%), indicating structural complexity and suggesting a potential enhancement of FGFR2 expression or signaling dependency of MSGC (Fig. 3C).

Integrated clinical-molecular score demonstrated a hierarchical organization in which *FGFR2* alterations and *TP53* exhibited the highest biological relevance and clonal representation, whereas most additional variants clustered within moderate- or low-impact categories (Fig. 4).

A concurrent *TP53* alteration was identified at high clonal representation (VAF 27.5%), supporting its role in tumor progression and genomic instability in MSGC. Additional secondary alterations were detected at lower variant allele frequencies, including *FGF19* (VAF 8.6%), *ABL1* (VAF 7.3%), *NFS4* (VAF 4.9%), *KDM4A* (VAF 4.6%), *DNMT3A* (VAF 4.1%), and *PIK3CA* (VAF 3.3%), indicating cooperative events related to growth signaling, metabolic adaptation, and epigenetic regulation. The overall distribution of somatic alterations according to variant allele frequency and clinical-molecular score confirmed a tumor architecture dominated by FGFR2 pathway activation, accompanied by multiple secondary events that may support tumor evolution and biological heterogeneity. In the hierarchical visualization, primary drivers are represented as red circles, whereas secondary or cooperative alterations are shown in blue (Supplementary Fig. S2).

## Discussion

MSGC are biologically heterogeneous malignancies characterized by variable clinical behavior and limited systemic therapeutic options. Given the significant morbidity associated with advanced disease, the identification of actionable molecular alterations has become essential for supporting precision oncology strategies and precision medicine. Comprehensive genomic profiling using NGS enables the identification of several fusion events, but clinically relevant driver events that may guide targeted treatment selection [12].

The contribution of *SDHC*, a component of mitochondrial complex II, is most likely limited to secondary metabolic and redox alterations rather than direct oncogenic activation. Loss of succinate dehydrogenase function is associated with mitochondrial dysfunction, accumulation of succinate as an oncometabolite, stabilization of hypoxia-inducible pathways, and increased oxidative stress, which may indirectly promote epigenetic reprogramming, genomic instability, and tumor adaptation rather than acting as primary driver events [13–15]. In this context, the *SDHC-RPA1* rearrangement is best interpreted as a permissive alteration that enhances genomic plasticity and clonal evolution. Disruption of RPA1 function is expected to increase replication stress and impair DNA damage tolerance, potentially creating a state of increased dependence on cell cycle checkpoints and DNA damage response (DDR) signaling. Such conditions provide a biological rationale for potential sensitivity to *ATR*, *CHK1*, or *WEE1* inhibitors in clinical trial settings, rather than representing a direct therapeutic target [16–17].

The coexistence of multiple *FGFR2* structural events in this patient further supports oncogene dependency and pathway amplification, reinforcing FGFR signaling as the dominant therapeutic target. The additional *AFG1L-ATF6B* fusion suggests activation of the ER stress and unfolded protein response (UPR) pathways, which may enhance tumor survival under oncogenic stress and contribute to adaptive resistance to targeted therapy [18]. Concurrent tumor suppressor alterations, including *TP53* loss and *NF1* inactivation, likely promote genomic instability and further enhance MAPK pathway signaling, facilitating tumor progression rather than initiating tumorigenesis [19], these adaptive mechanisms may have clinical implications for treatment durability and support the consideration of combination strategies targeting downstream MAPK signaling or cellular stress responses.

Overall, these cases illustrate two distinct yet clinically relevant molecular scenarios in major salivary gland carcinoma (MSGC): (i) MAPK-driven disease mediated by a *BRAF* fusion within a permissive background of genomic instability and (ii) FGFR2-dependent oncogenic signaling driven by activating receptor fusions. From a translational perspective, these findings support the prioritization of targeted therapy according to the dominant driver alteration, including MEK inhibition or RAF-MEK pathway targeting for *BRAF* fusion-positive tumors and selective FGFR inhibition for FGFR2-rearranged disease [20–22]. In addition, the presence of permissive alterations associated with replicative stress or endoplasmic reticulum stress suggests potential mechanisms of adaptive resistance and highlights biological vulnerabilities that may inform enrollment in clinical trials exploring DNA damage response inhibitors or rational combination strategies [23]. Importantly, these cases demonstrate that

comprehensive genomic profiling can identify actionable mutations and fusion events in rare MSGC subtypes. Such integrative molecular characterization is essential for refining prognostic assessment, enabling biomarker-driven therapy selection, and optimizing clinical trial matching for patients with fusion-driven MSGC.

## Abbreviations

**ABL1:** Abelson tyrosine kinase 1

**AFG1L:** AFG1 Like ATPase

**ATF6B:** Activating Transcription Factor 6 Beta

**ATR:** Ataxia Telangiectasia and Rad3-related

**BRAF:** B-Raf proto-oncogene, serine/threonine kinase

**CE:** Cancer Evidence

**CHK1:** Checkpoint kinase 1

**DDR:** DNA Damage Response

**DNMT3A:** DNA Methyltransferase 3 Alpha

**ER:** Endoplasmic Reticulum

**ERK –** Extracellular signal-regulated kinase

**ETV6:** ETS Variant Transcription Factor 6

**FGF19:** Fibroblast Growth Factor 19

**FGFR2:** Fibroblast Growth Factor Receptor 2

**HR:** High Risk/Frequency

**IP:** Interaction Pathway

**KDM4A:** Lysine Demethylase 4A

**LOC:** Genomic locus (uncharacterized region)

**MAPK:** Mitogen-Activated Protein Kinase

**MEK:** MAPK/ERK kinase

**NGS:** Next-Generation Sequencing

**NF1:** Neurofibromin 1

**NP:** Network Position

**OPRK1:** Opioid Receptor Kappa 1

**PI3K:** Phosphoinositide 3-kinase

**PIK3CA:** Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

**RPA1:** Replication Protein A1

**RAS:** Rat sarcoma viral oncogene homolog

**SC:** Strong Cancer driver

**SDHC:** Succinate Dehydrogenase Complex Subunit C

**SGC:** Salivary Gland Carcinoma

**SGT:** Salivary Gland Tumor

**TP53:** Tumor Protein p53

**UPR:** Unfolded Protein Response

**VAF:** Variant Allele Frequency

**WHO:** World Health Organization

## **Declarations**

### **Ethics Approval and Consent to Participate**

All procedures performed in this study were conducted in accordance with the ethical standards of the Declaration of Helsinki and its subsequent amendments. Given the retrospective nature of the study and the use of data generated during routine clinical practice, formal ethics committee approval and individual informed consent for inclusion in this analysis were waived in accordance with the applicable local regulations. All participating institutions were informed of the study and provided the necessary institutional authorizations. The authors are accountable for all aspects of the work and ensure that any questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

### **Consent for Publication**

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent amendments. Written informed consent for participation was obtained from the patient as part of routine clinical care protocol and gave written informed consent for their personal or clinical details along with any identifying images to be published in this study. Given the retrospective nature of the analysis, formal ethics committee approval and individual informed consent for inclusion in this study were waived in accordance with the applicable local regulations. All participating institutions were informed of the study and provided the necessary institutional authorization.

## **Consent for Publication**

Written informed consent was obtained from the patient for participation as part of routine clinical care, including explicit consent for publication of clinical data and any accompanying images. A copy of the written consent is available for review by the editor of this journal upon request.

## **Competing interests**

M.G. reports as Principal Investigator in clinical trials: MSD, BMS, Novartis, Roche, Astellas, Deciphera, PPD, IQVIA, Bayer, Principia, Covance, Daiichi-Sankyo, Basilea, PRA-Exelisis, Syneos, Zimeworks. The other authors do not declare any conflicts of interest.

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## **Authors contributions**

**Conceptualization:** J.V-T.; D.I.M. **Methodology:** F.S. Ch.; M.A.S.; M.E.A.; C.SM. A.; M.G.V.

**Investigation:** F.S.B.; M.N.B.; P. R de la T.; W.F.; N.C.; T.M.F.; R.L.F. **Writing-original draft:** A.R.T.; H.B.

**Writing-review and editing:** J.A.G.; M.G.

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## Figures

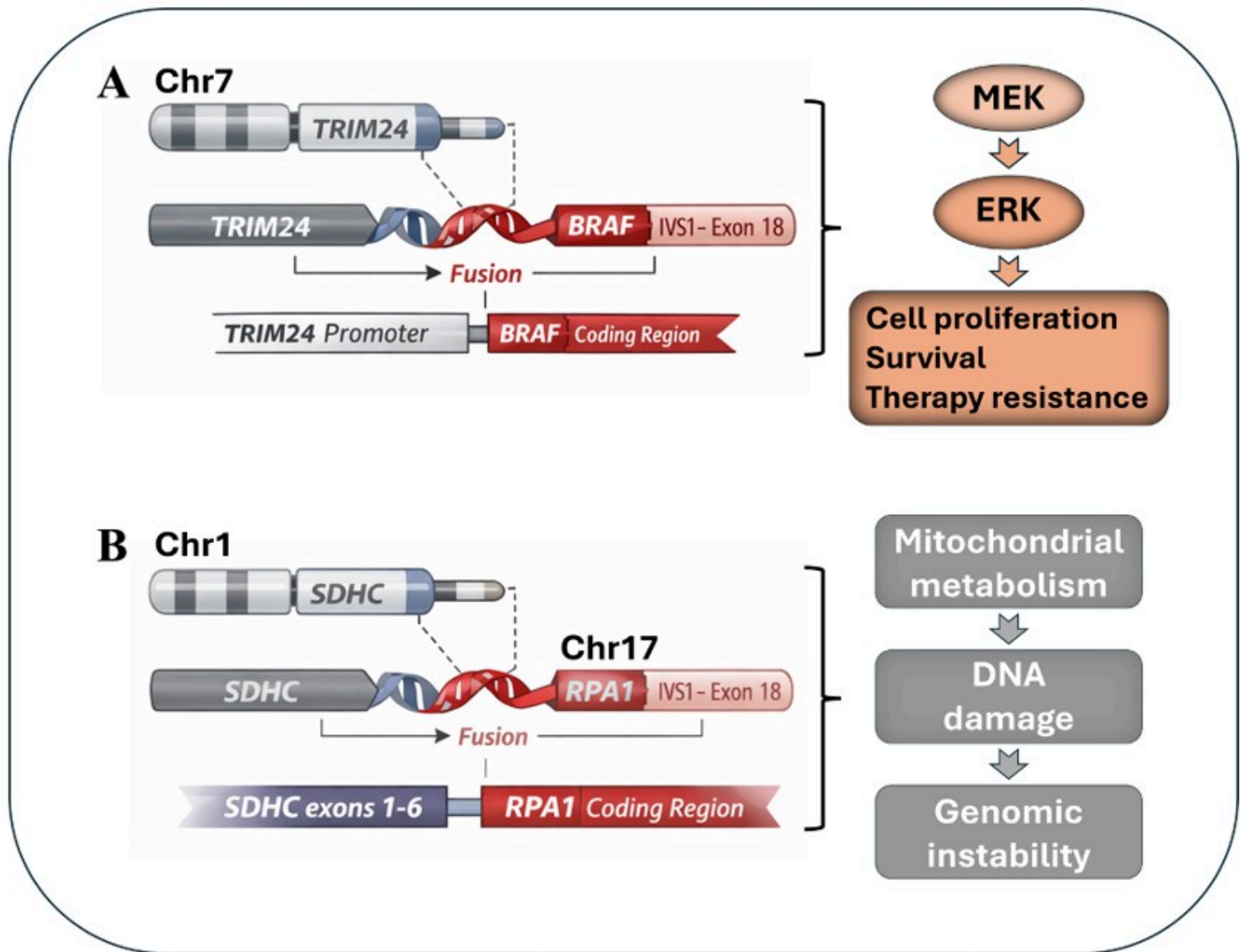


Figure 1

**Structural Gene fusions and predicted functional impact in patient 1 with MSGC.** (A) *TRIM24-BRAF* fusion on chromosome 7, which places the *TRIM24* promoter upstream of the *BRAF* coding region (including exon 18), leading to constitutive *BRAF* expression and activation of the MAPK (MEK-ERK) pathway, promoting tumor cell proliferation and survival. (B) *SDHC-RPA1* fusion involving chromosomes 1 and 17, joining *SDHC* exons 1-6 to the *RPA1* coding region. This rearrangement is predicted to link mitochondrial dysfunction with impaired DNA replication and repair, resulting in metabolic stress, genomic instability, and tumor progression.

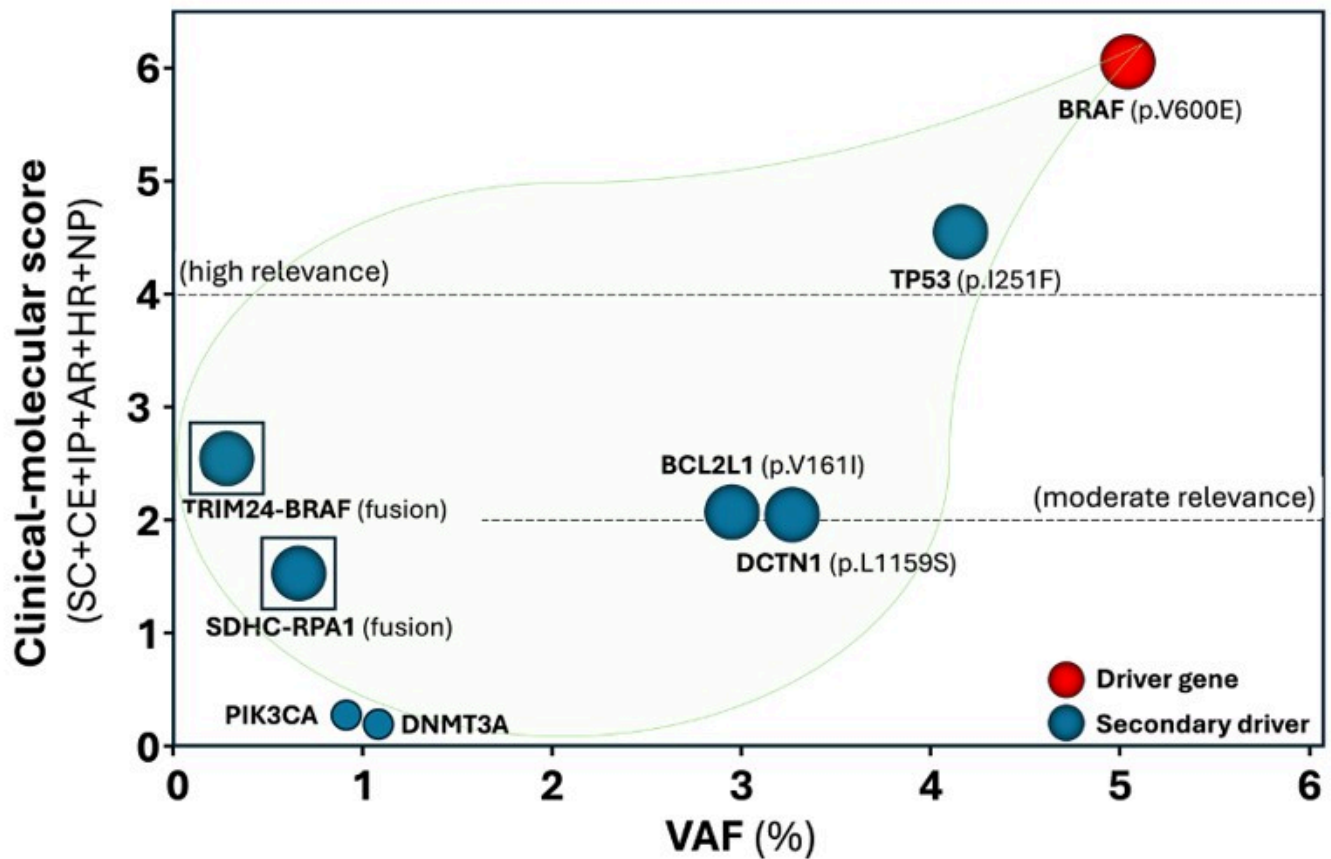


Figure 2

### Clinical-molecular stratification and clonal hierarchy of genomic alterations in patient 1 with MSGC.

Scatter plot showing the relationship between variant allele frequency (VAF, %) and the integrated clinical-molecular score (SC + CE + IP + AR + HR + NP). Each circle represents a genomic event (red, dominant drivers; blue, secondary or cooperative changes). *BRAF* (p.V600E) and *TP53* (p.L251F) clustered in the high-impact region, consistent with major oncogenic drivers. *BCL2L1* and *DCTN1* showed moderate impact at intermediate VAF, *PIK3CA* and *DNMT3A* show low VAF (~1%). Structural rearrangements as *TRIM24-BRAF* and *SDHC-RPA1* were detected at low VAF with lower scores, suggesting subclonal cooperation. Horizontal dashed lines indicate thresholds for moderate (~2) and high (~4) clinical-molecular relevance. This distribution supports a hierarchical tumor architecture characterized by high-impact clonal drivers and multiple lower-impact secondary alterations. *TRIM24*: Tripartite Motif containing 24, *BRAF*: Serine threonine kinase, *SDHC*: Succinate Dehydrogenase Complex Subunit C, *RPA1*: Replication Protein A1; SC (Strong Cancer driver), CE (Cancer Evidence), IP (Interaction Pathway), AR (Actionability/ treatments), HR (High risk/frequency), NP (Network Position).

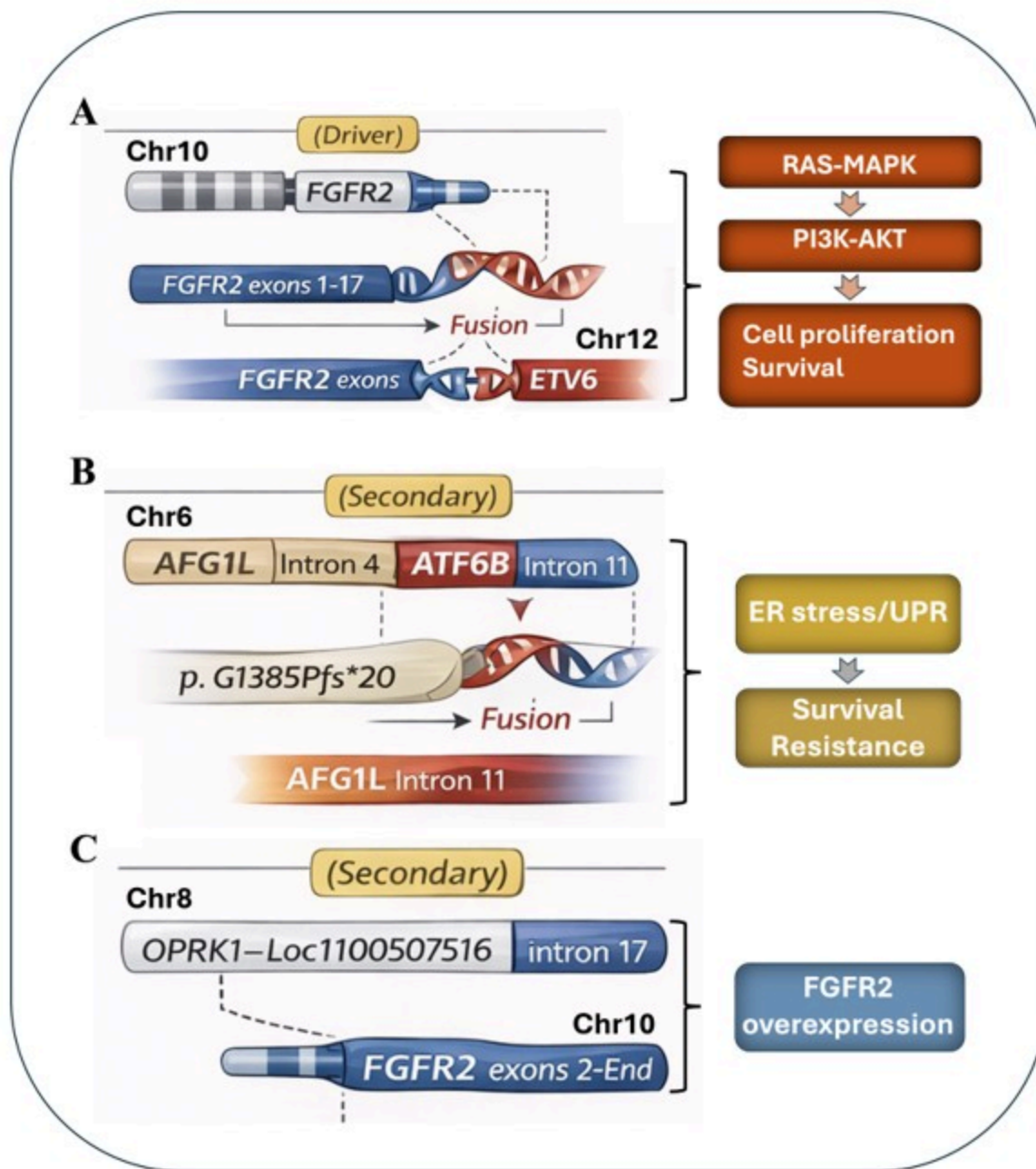


**Figure 3**

***FGFR2* structural rearrangements and cooperative Gene fusion events in patient 2 with MSGC. (A)** Schematic representation of the *FGFR2-ETV6* fusion (chromosomes 10 and 12), retaining *FGFR2* exons 1-17 and leading to constitutive activation of the RAS-MAPK and PI3K-AKT pathways, consistent with a primary oncogenic driver. **(B)**Diagram show the *AFG1L-ATF6B* rearrangement on chromosome 6, associated with a truncating event and predicted activation of the ER stress/unfolded protein response (UPR) pathway. **(C)** Intergenic *OPRK1-LOC1100507516-FGFR2* rearrangement predicted to enhance *FGFR2* expression and signaling. Together, these alterations support a hierarchical architecture in which *FGFR2* activation represents the dominant driver, and additional rearrangements act as secondary cooperative events contributing to tumor adaptation. *FGFR2*: Fibroblast growth factor receptor 2, *ETV6*: ETS Variant

Transcription Factor 6, *AFG1L*: AFG1 Like ATPase, *ATF6B*: Activating Transcription Factor 6 Beta, *OPRK1*: Opiod receptor kappa 1. LOC100507516.

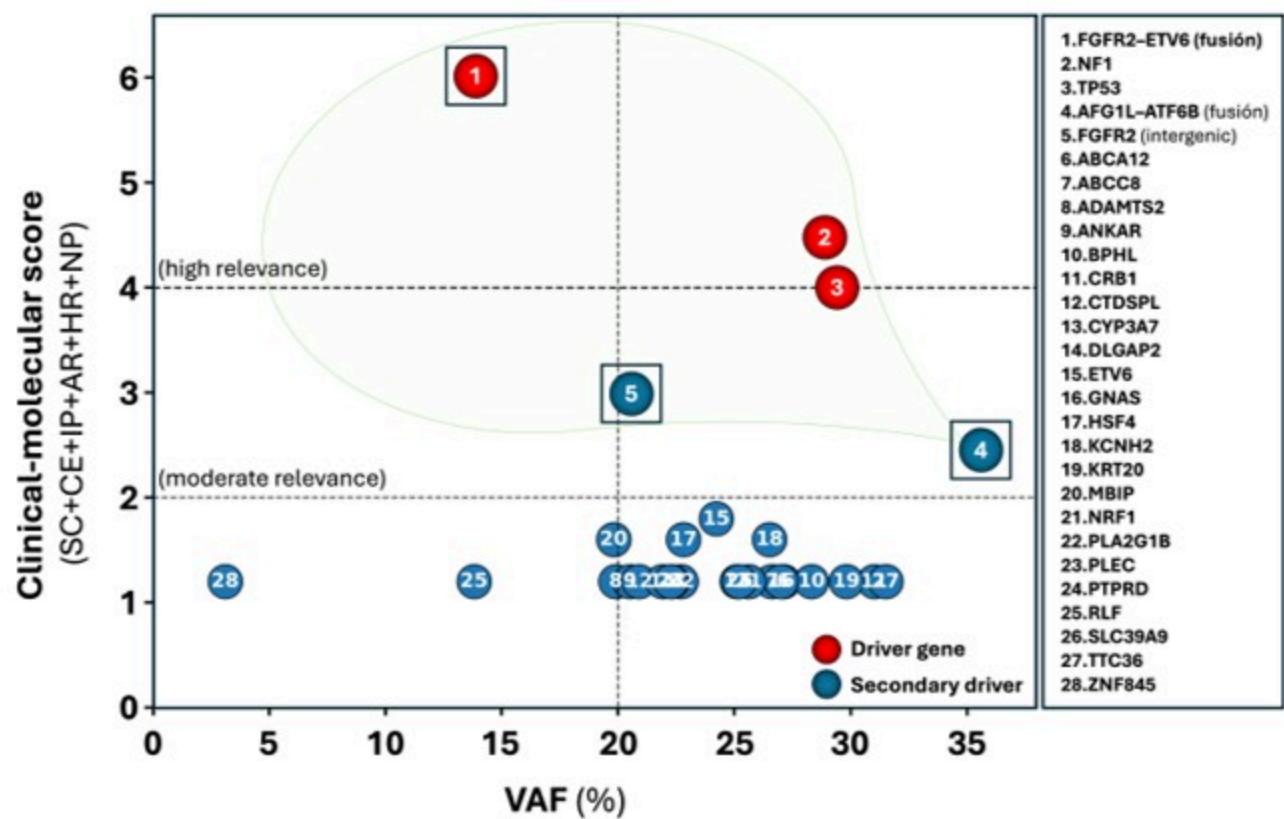


Figure 4

**Integrated clinical-molecular stratification and clonal hierarchy of genomic alterations in patient 2 with MSGC.** Scatter plot illustrating the relationship between variant allele frequency (VAF, %) and the integrated clinical-molecular score (SC + CE + IP + AR + HR + NP). Each numbered circle represents a genomic alteration (red, dominant drivers; blue, secondary, or cooperative events). The *FGFR2-ETV6* fusion showed the highest clinical-molecular impact, consistent with a primary oncogenic driver, whereas *NF1* and *TP53* also clustered within the high-relevance region (score  $\geq 4$ ). The *AFG1L-ATF6B* fusion and an intergenic *FGFR2* alteration displayed moderate relevance, whereas most additional variants (6-28) were located in the low-impact region, despite variable VAF, consistent with secondary or passenger events. Horizontal dashed lines indicate thresholds for moderate ( $\approx 2$ ) and high ( $\approx 4$ ) clinical-molecular relevance, and the vertical line (VAF  $\approx 20\%$ ) separates lower from higher clonal representation. The overall distribution supports a hierarchical tumor architecture driven by a limited number of high-impact events on a background of cooperative alteration. SC (Strong Cancer driver), CE (Cancer Evidence), IP (Interaction Pathway), AR (Actionability/ treatments), HR (High risk/frequency), NP (Network Position).