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Co-occurrence of Germline TP53 p.R337H and XAF1 p.E134* in Cancer- Predisposition Families in Central-West Brazil

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Article

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Abstract

Background

The germline *TP53* p.R337H founder variant and *XAF1* p.E134* have been described on an extended 17p13 haplotype in Brazil, but data from Central-West Brazil remain limited. We evaluated their co-occurrence in *TP53* p.R337H carriers from Goiás.

Methods

This cross-sectional study comprised 448 individuals referred for hereditary cancer evaluation (Cohort 1) and 101 consecutive pediatric oncology patients (Cohort 2), incorporating cascade family screening. *TP53* p.R337H carriers and relatives underwent *XAF1* p.E134* genotyping. Familial segregation and co-occurrence patterns were evaluated descriptively, and cancer-associated index cases were compared with a published newborn reference cohort.

Results

Co-occurrence was detected in 63/67 carriers (94.0%) in Cohort 1 and 26/27 (96.3%) in Cohort 2, totaling 89/94 (94.7%; 95% CI, 88.0- 98.3%). Five carriers had *TP53* p.R337H alone. Both variants co-occurred in 15/16 families (93.8%), whereas none of 103 *TP53*-negative relatives carried *XAF1* p.E134*. Co-occurrence among cancer-associated index cases exceeded that in newborns (OR = 6.78; 95% CI, 1.20 to Inf; *P* = 0.0265).

Conclusions

TP53 p.R337H and *XAF1* p.E134* frequently co-occur in Goiás cancer-predisposition families. These findings extend evidence of their regional association. Discordant carriers suggest haplotypic heterogeneity, warranting molecular phasing and larger studies to determine the clinical significance of *XAF1* p.E134* as a potential modifier of *TP53*-associated cancer risk.

Introduction

Li-Fraumeni syndrome (LFS; OMIM #151623) is a rare autosomal dominant hereditary cancer predisposition disorder associated with pathogenic or likely pathogenic germline variants in the *TP53* tumor suppressor gene.[1,2] The syndrome is characterized by high but variable cancer penetrance and a broad tumor spectrum, including premenopausal breast cancer, soft-tissue and bone sarcomas, adrenocortical tumors, brain tumors, and other malignancies frequently diagnosed at an early age.[3,4]

In Brazil, the *TP53* c.1010G > A (p.Arg337His; p.R337H) founder variant, located in the tetramerization domain of p53 rather than its DNA-binding domain, has an estimated carrier frequency of approximately 0.3% in Southern and Southeastern Brazil.[5,6] Although p.R337H exhibits hypomorphic properties, with reduced p53 activity, carriers show considerable variability in cancer susceptibility and clinical presentation, suggesting that additional genetic factors may influence disease expression.[6]

In this context, *XAF1* p.E134* has emerged as a potential genetic modifier of the *TP53* p.R337H-associated phenotype. Pinto et al. (2020) identified the *XAF1* c.400G > T (p.Glu134Ter; p.E134*) nonsense variant on chromosome 17p13.2 as part of an extended haplotype containing *TP53* p.R337H in Brazilian families.[7] In the study, the extended haplotype was associated with an increased frequency of sarcoma as the first malignancy (OR = 6.33; *P* = 0.003) and multiple primary malignancies (OR = 4.53; *P* = 0.006).[7]

The geographic distribution of the extended haplotype involving *TP53* p.R337H and *XAF1* p.E134* remains poorly characterized outside Southern and Southeastern Brazil. Recent evidence suggests that historical population expansion and migration contributed to the dissemination of the *TP53* p.R337H founder variant beyond these regions.[8] However, investigating the frequency of its co-occurrence with *XAF1* p.E134* in Central-West Brazil, more specifically, in Goiás may provide further insight into the geographic distribution of the founder haplotype and its potential clinical relevance.

We conducted a two-cohort study with cascade genetic screening in the state of Goiás, Central-West Brazil, to determine the frequency of co-occurrence of *TP53* p.R337H and *XAF1* p.E134* among *TP53* p.R337H carriers identified through hereditary cancer genetics and pediatric oncology settings and their relatives, and to descriptively characterize the tumor spectrum observed among affected carriers.

Methods

Study design and overview

This was a two-cohort cross-sectional study with systematic cascade genetic screening, conducted in Goiás state, Central-West Brazil. The cohorts were recruited and genotyped independently without overlapping participants, using shared laboratory infrastructure and analytical protocols at the Centre of Excellence in Human Genetics (CEGH-ICB/UFG). The study was conducted in accordance with the ethical approval granted under the project "Molecular analysis of alterations in the *BRCA1/2* and *TP53* genes in breast carcinoma patients treated by the Brazilian Public Health System (SUS)" (CAAE 50626315.6.0000.5078) and with the Declaration of Helsinki. Adult participants provided written informed consent (TCLE) following pre-test genetic counseling. For minors, a parent or legal guardian provided written informed consent, and participant assent was obtained when applicable.

Cohort 1: Hereditary Cancer Genetics (CEGH-ICB/UFG)

Cohort 1 was conducted at the Centre of Excellence in Human Genetics (CEGH-ICB), Federal University of Goiás (UFG), from January 2022 to July 2026. Individuals referred from the Clinics Hospital - UFG (HC-UFG), Araújo Jorge Cancer Hospital (HCAJ), and other public health units of the Brazilian Unified Health System (SUS) for evaluation of hereditary cancer predisposition were eligible.

Patient screening was based on family history and clinical criteria from the National Comprehensive Cancer Network (NCCN) guidelines for hereditary cancer predisposition assessment, including age at tumour onset (≤ 45 years), cancer type, multiple primary tumours in the same individual, multiple family members with the same or related types of cancer, and cancer occurring across more than one generation within the same family.

Proband samples (n = 448) were initially screened using a multigene next-generation sequencing (NGS) panel that included *TP53*. *TP53* p.R337H carriers and their first- and second-degree relatives were invited for cascade screening. A total of 139 relatives underwent *TP53* p.R337H genotyping. Of the *TP53* p.R337H-negative relatives, 64 were also genotyped for *XAF1* p.E134* and served as a reference group.

Cohort 2: Pediatric Oncology (HCAJ)

Cohort 2 was conducted at the Araújo Jorge Cancer Hospital (HCAJ), Association for Combating Cancer in Goiás (ACCG), from November 2024 to May 2026. Consecutive pediatric oncology patients aged < 18 years with confirmed cancer diagnoses were prospectively enrolled (n = 101). Germline *TP53* p.R337H status was assessed by targeted qPCR in all enrolled patients. First- and second-degree relatives of *TP53* p.R337H-positive probands were invited for cascade screening. A total of 70 relatives underwent *TP53* p.R337H genotyping. Among relatives who tested negative for *TP53* p.R337H, 39 were additionally genotyped for *XAF1* p.E134* and included as a reference group. Complete three-generation pedigrees were constructed for all positive families.

DNA extraction

Germline DNA was extracted from peripheral blood leukocytes; detailed extraction and quality-control procedures are provided in the Supplementary Methods.

Genotyping and qPCR analysis

Germline *TP53* p.R337H was genotyped by targeted qPCR with a commercial allele-specific assay. Positive individuals were subsequently genotyped for *XAF1* p.E134* using an in-house allele-specific qPCR assay.

Primers and allele-specific probes were designed using the NCBI Primer-BLAST and GeneRunner platforms. Genotypes were determined by allelic discrimination using QuantStudio Design & Analysis

Software. Assay composition, primer and probe sequences, reaction conditions, and qPCR quality-control procedures are detailed in the Supplementary Methods.

NGS multigene panel (Cohort 1)

Cohort 1 probands were screened using an Ion Torrent-based multigene NGS panel including *TP53* and 14 additional hereditary cancer-associated genes. Variant identification was performed using Ion Reporter Software. Library preparation and sequencing procedures are detailed in the Supplementary Methods.

Statistical analysis

The primary analysis was descriptive. Frequencies of *XAF1* p.E134* among *TP53* p.R337H carriers were summarized as absolute numbers and percentages for each cohort and for the combined dataset, with exact 95% confidence intervals calculated using the Clopper-Pearson method. Analyses included index cases and relatives identified through cascade screening. *TP53* p.R337H-negative relatives who underwent *XAF1* p.E134* genotyping were described separately. Continuous variables were summarized using median, interquartile range, and range, when applicable. Analyses were performed using GraphPad Prism version 10.1.0 (GraphPad Software, Boston, MA, USA).

To evaluate enrichment of *XAF1* p.E134* among cancer-associated *TP53* p.R337H carriers, genotype frequencies were compared with those of an external newborn reference cohort using a one-sided Fisher's exact test. The odds ratio (OR) was also calculated via Conditional Maximum Likelihood Estimation, accompanied by its 95% CI to evaluate whether, among *TP53* p.R337H carriers, the odds of concomitantly presenting the *XAF1* p.E134* variant were higher in the cancer patient group compared with the newborn cohort used as reference. Because three Cohort 1 index cases could not undergo direct molecular testing, this comparison was considered exploratory. For these cases, family-level co-occurrence status was inferred from segregation among genotyped relatives. This cohort was obtained from the study by Pinto et al. (2020), which evaluated 42,438 newborns from Southern Brazil and identified 147 *TP53* p.R337H carriers, of whom 101 (68.7%) also presented the *XAF1* p.E134* alteration.[7] A P value < 0.05 was considered statistically significant. Statistical analyses were performed using R software, version 4.6.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Screening outcomes and variant frequencies

The overall study flow, including primary screening, cascade testing, and *TP53* p.R337H/*XAF1* p.E134* co-occurrence across both cohorts, is summarized in Fig. 1.

CEGH-ICB/UFG = Centre of Excellence in Human Genetics, Institute of Biological Sciences, Federal University of Goiás; HCAJ = Araújo Jorge Cancer Hospital; NGS = next-generation sequencing; qPCR =

quantitative polymerase chain reaction.

Cohort 1 (CEGH-ICB/UFG - hereditary cancer genetics).

Among the probands screened, 13 families with *TP53* p.R337H-positive members were identified, with co-occurrence of *TP53* p.R337H and *XAF1* p.E134* in 12 (92.3%). Of the 13 index cases, 10 underwent direct genotyping and all carried both variants; the remaining three could not be tested because of death or refusal, and family-level status was assessed using genotyped relatives. Cascade screening identified 57 additional *TP53* p.R337H carriers, of whom 53 (93.0%; 95% CI, 83.0%-98.1%) also carried *XAF1* p.E134*. Discordant carriers were identified in Families 1, 9, and 11; in Family 11, all *TP53* p.R337H-positive individuals were *XAF1* p.E134*-negative. Overall, 63 of 67 molecularly confirmed *TP53* p.R337H carriers (94.0%) carried both variants. None of the 64 *TP53* p.R337H-negative relatives tested carried *XAF1* p.E134*.

Cohort 2 (HCAJ - pediatric oncology).

Of 101 pediatric oncology patients screened, three carried *TP53* p.R337H. Cascade screening identified 24 additional *TP53* p.R337H carriers, of whom 23 (95.8%; 95% CI, 78.9%-99.9%) also carried *XAF1* p.E134*. One relative carried *TP53* p.R337H without *XAF1* p.E134* in Family 7. Pedigrees of the families included in both cohorts, showing cancer history and segregation of *TP53* p.R337H and *XAF1* p.E134*, are presented in Supplementary Figure S1. Overall, 26 of the 27 molecularly confirmed *TP53* p.R337H carriers (96.3%) in Cohort 2 carried both variants. None of the 39 *TP53* p.R337H-negative relatives tested carried *XAF1* p.E134*.

Overall, across both cohorts, 89 of 94 (94.7%; 95% CI, 88.0%–98.3%) confirmed *TP53* p.R337H carriers also carried *XAF1* p.E134*, as shown in Table 1.

Table 1

Distribution of *TP53* p.R337H and *XAF1* p.E134* variant co-occurrence by cohort and screening type.

Cohort 1 - Hereditary cancer genetics			
Study population	**Screened, n**	***TP53* p.R337H-positive, n**	**Both variants, n/N (%)**
Probands (primary screening)	448	10*	10/10 (100.0)
Relatives (cascade screening)	139	57	53/57 (93.0)
Total confirmed carriers	—	67	63/67 (94.0)
TP53 p.R337H-negative relatives	64	0	0/64 (0.0)†
Families with confirmed carriers	13*	13 families	12/13 (92.3)
Cohort 2 - Pediatric oncology			
Study population	**Screened, n**	**TP53 p.R337H-positive, n**	**Both variants, n/N (%)**
Patients (primary screening)	101	3	3/3 (100.0)
Relatives (cascade screening)	70	24	23/24 (95.8)
Total confirmed carriers	—	27	26/27 (96.3)
TP53 p.R337H-negative relatives	39	0	0/39 (0.0)†
Families with confirmed carriers	3	3 families	3/3 (100.0)
Combined analysis			
Study population	**Screened, n**	**TP53 p.R337H-positive, n**	**Both variants, n/N (%)**
Primary screening	549	13*	13/13 (100.0)
Cascade screening	209	81	76/81 (93.8)
Total confirmed carriers	—	94	89/94 (94.7)
TP53 p.R337H-negative relatives	103	0	0/103 (0.0)†
Families with confirmed carriers	16	16 families	15/16 (93.8)

*Thirteen families were represented in Cohort 1. Three index cases could not undergo direct molecular testing because of death or refusal. Family-level *TP53* p.R337H/*XAF1* p.E134* status was therefore

determined from available genotyped relatives. One of these families included *TP53* p.R337H-positive relatives who did not carry *XAF1* p.E134*.

† All relatives included in cascade screening underwent *TP53* p.R337H genotyping. Among relatives who tested negative for *TP53* p.R337H, only a subset was additionally genotyped for *XAF1* p.E134*: 64 of 82 in Cohort 1 and 39 of 46 in Cohort 2. Therefore, the *TP53* p.R337H-negative reference group includes only those individuals with available *XAF1* genotyping results.

Comparison of TP53 p.R337H/XAF1 p.E134* co-occurrence with an external reference cohort

In an exploratory analysis of the 16 cancer-associated index cases, including three Cohort 1 probands whose family-level status was inferred from segregation, 15 (93.8%) showed co-occurrence of *TP53* p.R337H and *XAF1* p.E134*. The discordant case was inferred from the segregation pattern observed in available relatives. All directly genotyped double-positive patients were heterozygous for both variants. Comparison of the *TP53* p.R337H + *XAF1* p.E134* genotype distribution between this group and the newborn reference population demonstrated a higher frequency of co-occurrence among cancer-associated index cases. Among *TP53* p.R337H carriers, the odds of concomitantly presenting the *XAF1* p.E134* variant were approximately 6.8 times higher in the cancer-associated group than in the reference population (OR = 6.78; 95% CI, 1.20 to Inf; $P = 0.0265$).

Geographic distribution

Most probands (14/16, 87.5%) were born in Goiás. Among the 75 of 81 *TP53* p.R337H-positive relatives with available birthplace information, 72 (96.0%) were also born in Goiás; the geographic distribution is shown in Fig. 2.

Numbers in parentheses indicate the number of relatives from each birthplace.

Tumor spectrum

Cohort 1 index cases showed a heterogeneous tumor spectrum, whereas all three Cohort 2 index cases had adrenocortical carcinoma (Table 2; Fig. 3).

Table 2
Clinicopathological characteristics of probands from *TP53* p.R337H-positive families.

Family	Index tumor	Age at diagnosis	Second primary tumor / recurrence	Sex	*XAF1* p.E134*
Cohort 1 - CEGH-ICB/UFG (Hereditary Cancer Genetics)					
1	Sarcoma of the arm	64 yr	Adenocarcinoma (age 65 year)	F	Positive
2	Pelvic osteosarcoma	46 yr	None	F	Positive
3	Prostate cancer	42 yr	Osteosarcoma	M	Positive
4	Prostate cancer	49 yr	None	M	Positive
5	Hormone receptor-positive, HER2-equivocal breast cancer	30 yr	None	F	Positive
6	Hormone receptor-positive, HER2-negative breast cancer	34 yr	None	F	Positive
9	Triple-negative breast cancer	44 yr	None	F	Positive
10	Hormone receptor-positive, HER2-positive breast cancer	33 yr	None	F	Positive
11	Breast sarcoma	66 yr	None	M	Not tested
12	Unknown breast cancer subtype	49 yr	None	F	Not tested
14	Triple-negative breast cancer	27 yr	Breast cancer (recurrence at age 50 year)	F	Positive
15	Hormone receptor-positive, HER2-equivocal breast cancer	56 yr	None	F	Positive
16	Choroid plexus carcinoma	At birth	None	F	Not tested
Cohort 2 - HCAJ (Pediatric Oncology)					
7	Adrenocortical carcinoma	13 yr	None	F	Positive
8	Adrenocortical carcinoma	7 yr	None	F	Positive
13	Adrenocortical carcinoma	10 mo	esophageal carcinoma (age 20 yr*)	F	Positive

mo = months; yr = years. Ages for Cohort 2 pediatric probands are approximate to the nearest year to protect participant privacy. *NGS* molecularly confirmed *TP53* p.R337H status in the 10 directly genotyped Cohort 1 index cases and by targeted qPCR in the three Cohort 2 index cases. For the three Cohort 1 index cases who could not undergo direct molecular testing, family-level *TP53* p.R337H status was established from available genotyped relatives.

*The patient who developed esophageal carcinoma at age 20 years was included in Cohort 2 because her index malignancy was adrenocortical carcinoma diagnosed at 10 months of age; the esophageal carcinoma represented a second primary malignancy.

Figure 3. Tumor spectrum among TP53 p.R337H carriers.

(A) Primary cancer type. (B) Secondary cancer type. Index cases and relatives are shown separately. *TP53* p.R337H status was inferred from family segregation for index cases from Families 11, 12, and 16.

Among 81 *TP53* p.R337H-positive relatives identified through cascade screening, eight (9.9%) had a history of cancer, and 73 (90.1%) had no reported cancer at last follow-up; tumor types among affected relatives are detailed in Table 3. Among cancer-free relatives with available age data (n = 70), the median age at last follow-up was 38.5 years (IQR, 22.25–54.75; range, 4–81).

Table 3
Cancer diagnoses among *TP53* p.R337H-positive relatives identified through cascade screening.

Family	Tumor type	Age at diagnosis	Second primary tumor / recurrence	Relationship to proband	Sex	*XAF1* p.E134*
1	Breast cancer	52	None	Daughter	Female	Positive
4	Lung cancer	54	None	Sister	Female	Positive
6	Breast cancer	36	Recurrence	Sister	Female	Positive
7	Kidney cancer	62	None	Aunt	Female	Positive
7	Uterine cancer	58	None	Aunt	Female	Positive
7	Breast cancer	42	Breast sarcoma (second primary, same breast, at age 47 year)	Mother	Female	Positive
12	Gastric cancer	62	None	Brother	Male	Positive
13	Breast cancer	45	None	Aunt	Female	Positive

Discussion

High co-occurrence and haplotypic context

The high *TP53* p.R337H/*XAF1* p.E134* co-occurrence observed here extends findings from Southern and Southeastern Brazil. Pinto et al. (2020) identified both variants in 157 of 199 cancer-affected *TP53* p.R337H carriers (78.9%) and in 101 of 147 newborn carriers (68.7%), and characterized an extended 17p13 haplotype containing both variants.[7]

Earlier haplotype studies supported a shared ancestral origin of the Brazilian *TP53* p.R337H founder allele, with evidence pointing to a Portuguese-Iberian origin,[5,9] while subsequent studies described its demographic expansion across Brazil[8] and the existence of multiple haplotypic backgrounds.[10] Thus, the high co-occurrence observed in Goiás is consistent with the geographic dissemination of the founder allele, although direct comparisons between studies should consider differences in geographic origin, recruitment, and family-based ascertainment.

Five *TP53* p.R337H carriers lacked *XAF1* p.E134*, demonstrating nonuniversal co-occurrence. Pinto et al. (2024) identified distinct *TP53* p.R337H haplotypes, including Hap1, which carried both *TP53* p.R337H and *XAF1* p.E134*, and Hap2, which lacked the *XAF1* variant.[10]

Accordingly, the discordant genotypes observed in this study may reflect haplotypic heterogeneity, including recombination between the loci or inheritance of *TP53* p.R337H on a different genetic background. However, because formal chromosomal phasing was not performed, the location of the two variants in cis and the molecular basis of these discordant cases cannot be established here.

XAF1 p.E134* enrichment among cancer-associated index cases

In this exploratory comparison, *XAF1* p.E134* co-occurrence was more frequent among cancer-associated *TP53* p.R337H index cases than in the published newborn reference cohort (93.8% vs 68.7%).[7] The estimated odds ratio indicated nearly seven-fold higher odds of *XAF1* p.E134* co-occurrence in the cancer-associated group compared with the reference population (OR = 6.78; 95% CI, 1.20 to Inf; $P = 0.0265$). However, this estimate should be interpreted cautiously given the small number of index cases, family-based ascertainment, geographic differences between the study populations, and the inclusion of three probands with family-level inferred status. This finding is consistent with the results reported by Pinto et al. (2020), who identified that the *XAF1* p.E134* variant is present in ~ 79% of the 199 evaluated heterozygous *TP53* p.R337H-carrier participants with cancer (OR = 1.70; 95% CI: 1.10 to Inf; $P = 0.022$).[7]

However, the small number of cancer-associated carriers (n = 16), particularly those with isolated p.R337H (n = 1), limited statistical precision and precluded stratified analyses by age or tumor type. In addition, the newborn sample used in the comparisons consists of individuals from Southern Brazil; therefore, the baseline frequency of *XAF1* p.E134* in *TP53* p.R337H carriers in that region may diverge

from that found in the Central-West region.[7] This potential geographical difference reinforces the need for large-scale genotyping of both variants in different Brazilian populations. Such an investigation will establish regional population reference frequencies, thus enabling a more precise assessment of the *XAF1* p.E134* variant enrichment.

Tumor heterogeneity among index cases

Among the 13 probands in Cohort 1, seven presented with breast cancer as their index malignancy. Receptor subtype data were available for six cases: two were triple-negative breast cancers (TNBC), two were hormone receptor-positive/HER2-equivocal, one was hormone receptor-positive/HER2-negative, and one was hormone receptor-positive/HER2-positive. This heterogeneous distribution contrasts with previous studies reporting a substantial proportion of HER2-positive breast cancers among carriers of germline *TP53* variants. Masciari et al. reported HER2 positivity in 63% of invasive breast carcinomas in a LFS cohort,[11] while Sandoval et al., in a Brazilian cohort enriched for *TP53* p.R337H carriers, observed HER2 positivity in 39.5% of invasive breast carcinomas.[12] However, differences in the variants represented, patient selection, and receptor characterization limit direct comparisons.

Furthermore, all three Cohort 2 probands, varying in age, presented with adrenocortical carcinoma (ACC), a characteristic childhood malignancy associated with germline *TP53* p.R337H in Brazil.[4,6] In a Brazilian series of 103 children with adrenocortical tumors, Mastellaro et al. reported that most cases were diagnosed during early childhood, although a smaller proportion occurred after 5 years of age.[13] Similarly, Wasserman et al. demonstrated an age-dependent distribution of germline *TP53* variants among children with ACC, highlighting the importance of age and genetic background in its clinical presentation.[14] The youngest proband in our cohort subsequently developed esophageal carcinoma at age 20, representing a second primary malignancy. This observation is consistent with the lifelong cancer predisposition and occurrence of multiple malignancies described in families carrying *TP53* p.R337H.[15] However, the contribution of *XAF1* p.E134* to this individual's clinical course cannot be established from a single case.

The results of our cohort are consistent with previous evidence that factors beyond the founder variant itself influence tumor onset among *TP53* p.R337H carriers, as Surakhy et al. have identified.[16] These findings support the involvement of additional genetic modifiers in the clinical heterogeneity of ACC. Although Pinto et al. proposed *XAF1* p.E134* as a modifier of cancer susceptibility in *TP53* p.R337H carriers, their study did not establish an association between this variant and age at ACC onset,[7] and our study was likewise unable to assess this association. Larger studies including carriers with and without *XAF1* p.E134*, together with other potential genetic modifiers, are needed to investigate this possibility.

In this context, the biological relevance of *XAF1* p.E134* in carriers of *TP53* p.R337H is supported by functional evidence demonstrating that this variant may further compromise the residual transcriptional activity of the hypomorphic p53 R337H protein.[7] These findings provide a plausible biological basis for investigating *XAF1* as a modifier of cancer susceptibility in *TP53* p.R337H carriers. However, the small

number of breast cancer and ACC cases precluded meaningful assessment of associations between *XAF1* p.E134* and specific clinical phenotypes.

Cascade screening and relatives

Cascade screening extended genetic evaluation beyond affected probands and enabled the identification of at-risk relatives who may benefit from individualized counseling and surveillance. This is consistent with current recommendations supporting predictive testing in eligible relatives and surveillance tailored to the specific *TP53* variant and family history.[17]

Prospective evidence also suggests that structured biochemical and imaging surveillance may improve outcomes in germline *TP53* variant carriers.[18] However, the proportion of cancer-free relatives in our cohort should not be interpreted as an estimate of penetrance because of family-based ascertainment, age heterogeneity, and the lack of standardized longitudinal follow-up.

Limitations

Several limitations should be acknowledged. First, formal haplotype analysis to confirm whether *TP53* p.R337H and *XAF1* p.E134* are in cis has not been performed; our conclusions rest on co-occurrence rather than verified chromosomal phase. Second, the cross-sectional design with cascade ascertainment means that relatives were identified precisely because probands were positive, which may overestimate *XAF1* p.E134* frequency relative to population-based sampling. Third, the tumor spectrum data are observational and insufficient to estimate cancer risk or penetrance differences between carriers of both variants and carriers of *TP53* p.R337H alone. Fourth, *XAF1* p.E134* genotyping was not available for all index cases, and receptor subtype and clinical follow-up data were incomplete for some participants. Fifth, this study was conducted in a single state and may not represent the full diversity of *TP53* p.R337H carriers across Central-West Brazil.

These limitations notwithstanding, this study provides the first systematic documentation of *TP53* p.R337H/*XAF1* p.E134* co-occurrence in Goiás, Central-West Brazil, demonstrating a high frequency of co-occurrence while identifying carriers with discordant genotypes. These findings expand current knowledge of the geographic distribution of both variants and highlight the need for molecular phasing, larger population-based studies, and longitudinal follow-up to clarify the clinical significance of *XAF1* p.E134* in *TP53* p.R337H carriers.

Declarations

CRediT authorship contribution statement

L.M.S.: Conceptualization, Formal analysis, Investigation (Cohort 1: genotyping and cascade screening), Methodology, Writing – original draft, Writing – review & editing. **F.B.S.**: Conceptualization, Formal analysis, Investigation (Cohort 2: genotyping and pediatric case ascertainment), Methodology, Writing – original draft, Writing – review & editing. **K.M.V.**: Data curation, Investigation, Methodology, Validation,

Writing – review & editing. **P.F.F.S.**: Investigation, Resources, Supervision, Writing – review & editing. **R.M.G.**: Investigation, Methodology, Resources, Data curation, Writing – review & editing. **J.R.C.**: Investigation, Methodology, Resources, Validation, Writing – review & editing. **L.H.A.C.**: Methodology, Formal analysis, Writing – review & editing. **M.A.C.R.B.**: Investigation, Resources, Data curation, Writing – review & editing. **I.M.C.C.**: Investigation, Resources, Writing – review & editing. **B.E.F.N.**: Investigation, Resources, Writing – review & editing. **A.M.S.**: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **R.M.Q.**: Investigation, Methodology, Writing – review & editing. **H.R.L.**: Investigation, Resources, Writing – review & editing. **L.P.L.**: Investigation, Resources, Writing – review & editing. **J.M.C.B.**: Data curation, Investigation, Resources, Writing – review & editing. **V.S.R.F.**: Data curation, Investigation, Writing – review & editing. **W.D.O.**: Investigation, Resources, Writing – review & editing. **M.S.A.**: Methodology, Resources, Writing – review & editing. **R.M.S.R.**: Investigation, Resources, Writing – review & editing. **R.L.T.**: Investigation, Resources, Writing – review & editing. **A.R.G.**: Investigation, Resources, Writing – review & editing. **C.A.S.T.V.-C.**: Methodology, Resources, Writing – review & editing. **K.J.R.M.**: Investigation, Resources, Writing – review & editing. **A.M.S.L.**: Investigation, Resources, Writing – review & editing. **L.M.C.**: Investigation, Resources, Writing – review & editing. **R.L.M.**: Investigation, Resources, Writing – review & editing. **A.C.S.F.**: Investigation, Resources, Writing – review & editing. **L.N.M.**: Investigation, Resources, Writing – review & editing. **R.C.G.O.**: Investigation, Resources, Writing – review & editing. **L.V.S.C.D.**: Investigation, Resources, Writing – review & editing. **R.R.S.J.**: Investigation, Resources, Writing – review & editing. **E.P.S.-L.**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

ChatGPT (OpenAI) was used during manuscript preparation to assist with language editing, clarity, conciseness, and text organization. The tool was not used to generate research data or perform statistical analyses. All AI-assisted content was critically reviewed, edited, and verified by the authors, who assume full responsibility for the accuracy and integrity of the final manuscript.

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Author Contribution

L.M.S.: Conceptualization, Formal analysis, Investigation (Cohort 1: genotyping and cascade screening), Methodology, Writing – original draft, Writing – review & editing. F.B.S.: Conceptualization, Formal analysis, Investigation (Cohort 2: genotyping and pediatric case ascertainment), Methodology, Writing – original draft, Writing – review & editing. K.M.V.: Investigation, Data curation, Writing – review & editing. P.F.F.S.: Investigation, Resources, Writing – review & editing. R.M.G.: Investigation, Methodology, Resources, Data curation, Writing – review & editing. J.R.C.: Methodology, Investigation, Validation, Writing – review & editing. L.H.A.C.: Methodology, Formal analysis, Writing – review & editing. M.A.C.R.B.: Investigation, Resources, Data curation, Writing – review & editing. I.M.C.C.: Investigation, Resources, Writing – review & editing. B.E.F.N.: Investigation, Resources, Writing – review & editing. A.M.S.: Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. R.M.Q.: Investigation, Methodology, Writing – review & editing. H.R.L.: Investigation, Resources, Writing – review & editing. L.P.L.: Investigation, Resources, Writing – review & editing. J.M.C.B.: Investigation, Resources, Data curation, Writing – review & editing. V.S.R.F.: Investigation, Data curation, Writing – review & editing. W.D.O.: Investigation, Resources, Writing – review & editing. M.S.A.: Methodology, Resources, Writing – review & editing. R.M.S.R.: Investigation, Resources, Writing – review & editing. R.L.T.: Investigation, Resources, Writing – review & editing. A.R.G.: Investigation, Resources, Writing – review & editing. C.A.S.T.V.-C.: Resources, Methodology, Writing – review & editing. K.J.R.M.: Investigation, Resources, Writing – review & editing. A.M.S.L.: Investigation, Resources, Writing – review & editing. L.M.C.: Investigation, Resources, Writing – review & editing. R.L.M.: Investigation, Resources, Writing – review & editing. A.C.S.F.: Investigation, Resources, Writing – review & editing. L.N.M.: Investigation, Resources, Writing – review & editing. R.C.G.O.: Investigation, Resources, Writing – review & editing. L.V.S.C.D.: Investigation, Resources, Writing – review & editing. R.R.S.J.: Investigation, Resources, Writing – review & editing. E.P.S.-L.: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Methodology, Writing – review & editing.

Data Availability

The data underlying this article cannot be shared publicly due to the sensitive nature of germline genetic data and the need to protect participant privacy, in accordance with Brazilian Resolution CNS 466/2012 and the Brazilian General Data Protection Law (Lei Geral de Proteção de Dados - LGPD; Law No. 13,709/2018). Aggregated data supporting the findings are included in the article. Researchers seeking to collaborate or access de-identified data subsets for academic research purposes may submit a formal request to the corresponding author (elacerda@ufg.br), subject to approval by the UFG institutional research ethics committee (CEPAS/UFG).

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Figures

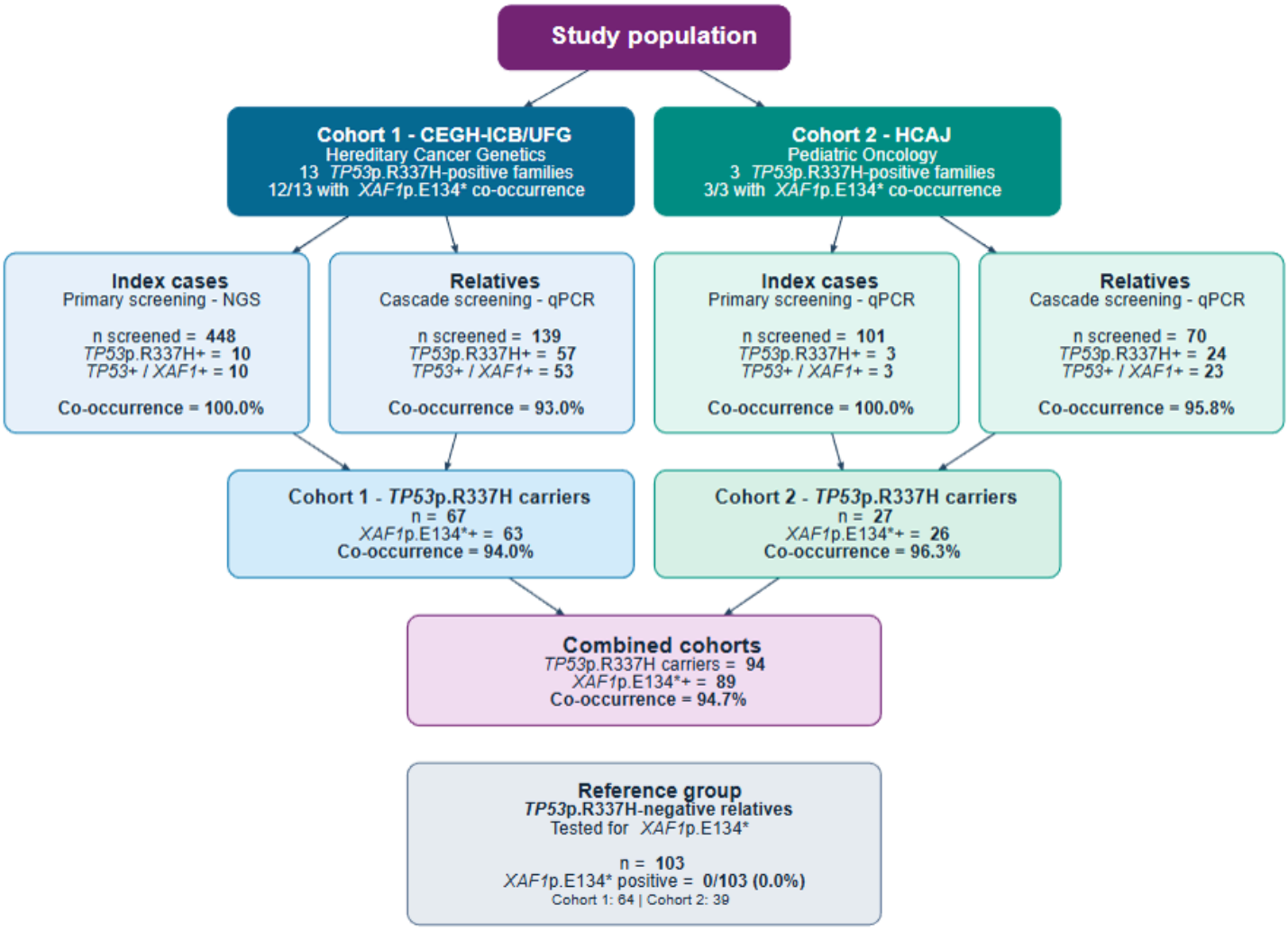

Figure 1

Flowchart of the study population and cohort composition.

CEGH-ICB/UFG = Centre of Excellence in Human Genetics, Institute of Biological Sciences, Federal University of Goiás; HCAJ = Araújo Jorge Cancer Hospital; NGS = next-generation sequencing; qPCR = quantitative polymerase chain reaction.

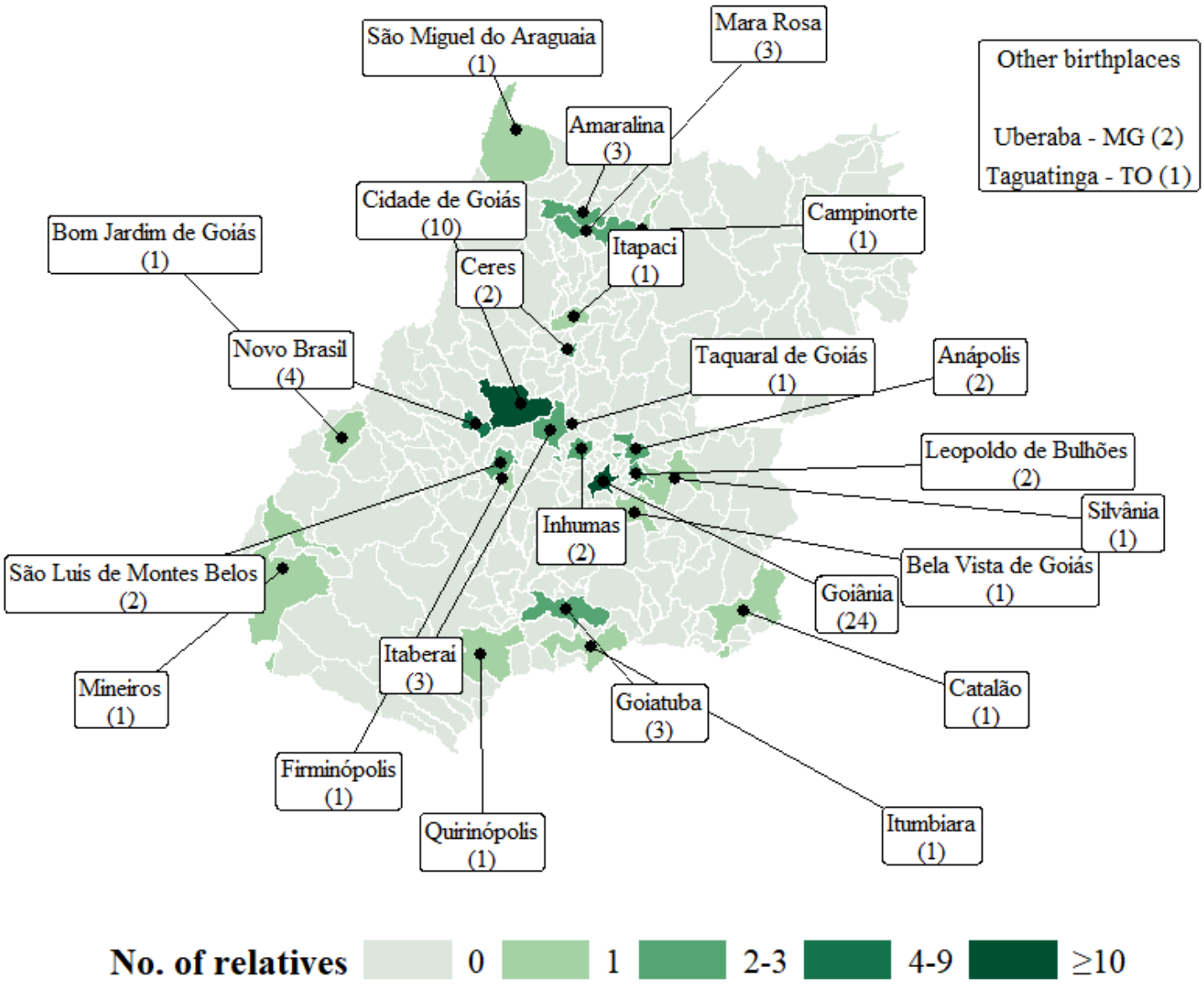

Figure 2

Geographic distribution of relatives according to place of birth.

Numbers in parentheses indicate the number of relatives from each birthplace.

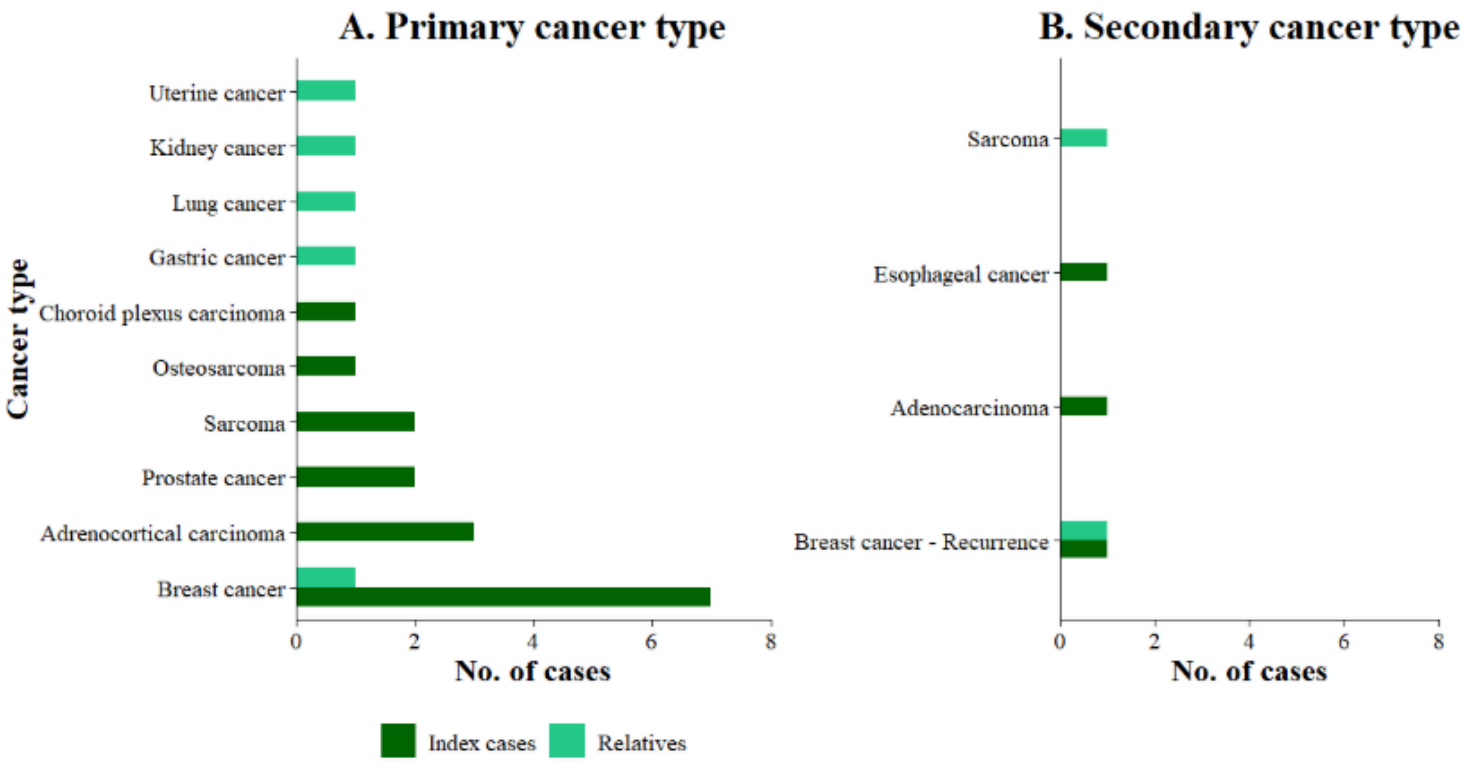

Figure 3

Tumor spectrum among *TP53*p.R337H carriers.

(A) Primary cancer type. (B) Secondary cancer type. Index cases and relatives are shown separately. *TP53* p.R337H status was inferred from family segregation for index cases from Families 11, 12, and 16.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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