CANCER GENETICS

original reports abstract

Tumor and Constitutional Sequencing for Neurofibromatosis Type 1

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PURPOSE *NF1* variants in tumors are important to recognize, as multiple mechanisms may give rise to biallelic variants. Both deletions and copy-neutral loss of heterozygosity (LOH) are potential mechanisms of *NF1* loss, distinct from point mutations, and additional genes altered may drive different tumor types. This study investigates whether tumors from individuals with neurofibromatosis type 1 (NF1) demonstrate additional gene variants and detects *NF1* second hits using paired germline and somatic sequencing. In addition, rare tumor types in NF1 may also be characterized by tumor sequencing.

MATERIALS AND METHODS Sequences of 529 cancer driver genes were analyzed in 6,381 tumors, yielding 391 *NF1*-mutated tumors in which *NF1* LOH analysis was performed. Driver genes were evaluated by tumor type including malignant peripheral nerve sheath tumors and gliomas.

RESULTS *NF1* LOH was seen in 133 of 391 tumor samples in the cohort. Individuals with NF1 had more prevalent copy-neutral LOH ($P < .0001$), suggesting somatic intrachromosomal recombination. Osteosarcoma in NF1 also had *NF1* LOH and additional p53 alteration. *NF1* second hit data from tumors were informative for inferring deleteriousness of missense variants that were conflicting in ClinVar, potentially helping to add to *NF1* annotation. Although criteria for evaluating germline and somatic variants are different, deleterious effects on *NF1* function may be shared.

CONCLUSION Sequencing of NF1-associated tumors demonstrated a spectrum of second hits in *NF1* and the prevalence of copy-neutral LOH. Future work may be aimed at further understanding of LOH mechanisms and strategies to mitigate tumor risk.

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common condition occurring in approximately 1 in 3,000 individuals.[1] Individuals with NF1 have an increased risk for tumor development with a predilection for specific subtypes compared with those without.[2,3] Some tumors, however, can be phenotypically similar. Malignant peripheral nerve sheath tumor (MPNST) is a well-known malignancy associated with NF1, but MPNST can also be unrelated to NF1.[4] *NF1* variants are seen in malignancies such as gliomas, but *NF1* variants may also lead to tumors that are not classically described in NF1.[5,6] Molecular characterization of tumors is therefore key to elucidating genetic origins underlying tumors. Loss of heterozygosity (LOH) occurs in NF1-related tumors and can occur via a deletion mechanism or through mitotic recombination, resulting in copy-neutral LOH.[7-9] Copy-neutral LOH has been seen in manifestations associated with NF1 including dystrophic scoliosis, tibial pseudarthrosis, juvenile myelomonocytic leukemia, and glomus tumors, suggesting that the somatic change has widespread implications.[10-13] Several unanswered questions remain, despite significant work in the field: What underlies the LOH observed? Are there strategies to mitigate second hits in NF1 or to understand the role of comutated genes? To characterize tumor-related genes in NF1-related tumors, large multigene sequencing platforms can be used to identify mutated *NF1* and possible homozygosity, suggesting potential opportunities to identify *NF1* loss and alterations in other oncogenes/tumor suppressors in tissue. If tumor sequencing and normal sequencing are performed simultaneously, variants detected can be reasonably identified in their sequence of occurrence.[7,14-16] Applied to a cohort of tumors, such sequencing can yield variants, genes, and potentially mutation mechanisms underlying tumor pathophysiology, particularly in tumors with limited burden of pathogenic variants. The data may also help with inferring pathogenicity attributed to variants in tumor and germline[17-20]

The study presents paired sequencing from samples from individuals with NF1, analyzes types of tumors

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Individuals with neurofibromatosis type 1 (NF1) are at increased risk for tumor development. Multiple mechanisms may lead to biallelic inactivation that drives these tumors. This study analyzed somatic and germline samples to determine the landscape of variation and the spectrum of second hits in *NF1*.

Knowledge Generated

Data from multigene sequencing demonstrated that the most prevalent type of second hit in tumors from individuals with constitutional NF1 was copy-neutral loss of heterozygosity. *NF1* variant annotation may benefit from analysis of paired normal-tumor data, including for osteosarcoma, a tumor rarely seen with NF1.

Relevance

Understanding of the mechanism of second hit acquisition in NF1 will be important for devising therapeutic and prophylactic strategies. With expanded use of tumor-normal sequencing, large amounts of data can be leveraged to understand pathogenesis and to interpret germline variants with conflicting or uncertain pathogenicity.

undergoing surgery, and explores the spectrum of second hits in *NF1*, including copy-neutral LOH. MPNST is analyzed for mutational patterns in NF1 and non-NF1. Data on NF1 and osteosarcoma are also presented. To examine the potential for tumor sequencing to assist with variant deleteriousness prediction, cases with two hits in *NF1* are analyzed, identifying missense variants that may play a pathogenic role.

MATERIALS AND METHODS

Genetic Evaluation of Tumors

Tumors were evaluated from 2015 to 2021, including a total of 6,168 tumors. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue. Capture-based next-generation sequencing was performed at the UCSF Clinical Cancer Genomics Laboratory using an assay (UCSF500) that targets the coding region of 529 cancer-related genes and selects introns from approximately 40 genes. Copy number variants were detected and visualized using Integrated Genome Viewer and Nexus Copy Number. Tumor samples contained at least 25% of tumor in samples with a standard quality read depth of 500×. Three hundred ninety-one tumors had a pathogenic or likely pathogenic (P/LP) *NF1* variant including nonsense, frameshift insertion or deletion, and splice donor/acceptor variants, and these were reviewed manually and compared with COSMIC,[20] ClinVar,[21] and gnomAD.[22] Missense variants were additionally annotated using several prediction tools including Combined Annotation-Dependent Depletion (CADD) scores.[22] Matched tumor-normal samples were paired from tumor and peripheral blood or buccal swab including 130 cases with matched tumor and normal samples, including 21 cases with germline *NF1* and 109 without germline *NF1*. Two hundred sixty-one cases were tumor-only submissions, and these tumors had *NF1* variants without immediate germline information. Nineteen additional cases were added on the basis of clinically diagnosed NF1 cases or cases where another genetic test demonstrated a P/LP *NF1* germline alteration. The median variant allele frequency (VAF) in tumor for *NF1* germline cases was 0.77 ± 0.19. In addition to *NF1*, comutated genes were also required to be P/LP with interpretation on the basis of standard laboratory criteria. Comutated variants had a median VAF of 0.35 ± 0.20. The Cancer Genome Atlas (TCGA) was examined for variant and tumor type comparisons.[23-25]

Mutation/LOH

Data corresponding to NF1 status were obtained from medical and genetic testing and the laboratory reports. Cases were analyzed for LOH at *NF1* (NM_001042492) and for other variants in any of the 529 genes. Copy-neutral LOH was assessed on the basis of the allelic ratio of polymorphic single nucleotide polymorphisms (SNPs) across 17q by the clinical laboratory team. The 133 cases that had LOH were categorized as LOH because of deletion or copy-neutral LOH, and these had higher median VAF compared with samples without LOH ($P < .0001$, Mann-Whitney U). Copy-neutral LOH status was determined according to technical laboratory guidance.[26] The status was also verified by a second laboratory genetic review of allele frequency and copy number.

NF1 germline cases were also analyzed for somatic tumor missense *NF1* variants; somatic variants were compared with ClinVar annotation. Eight missense variants in *NF1* were identified for review; four variants in tumor-only samples could not be further analyzed. D2632G carries a likely pathogenic interpretation on the basis of one submission in ClinVar with a germline R192*; the variants were too far apart to determine phase. I2615V was not annotated in ClinVar and was seen in a tumor with copy-neutral LOH and a germline splice variant. G629R and S82F were second *NF1* hits in tumor. The VAF in tumor for G629R and S82F was 0.27 and 0.82, respectively.

Data and Statistics

Genetic data were visualized using the ComplexHeatmap package in R.[27] Analyses were performed using R version 4.0.3.[28]

Ethics Declaration

This study was reviewed and approved by the University of California—San Francisco Institution Review Board (IRB, 20-33093). Informed consent was not required. It was not practicable to contact all the cases with tumors in this retrospective study. The research data were deidentified.

RESULTS

NF1 Alterations in Tumor Samples

Of 6,168 tumor samples, 391 tumors harbored P/LP *NF1* variants. Forty tumors were from individuals with constitutional NF1. The nervous system and skin were the most common anatomic sites for the *NF1*-altered tumors (Appendix Tables A1 and A2). There were 369 unique coding variants leading to 294 protein alterations. 85.4% were nonsense, frameshift insertion/deletion, or splice acceptor/donor variants, and 11.7% were pathogenic missense variants. Recurrent mutated sites affected NF1 residues Y2285, n = 19, and F1247, n = 18, which we confirmed as recurrent sites in the TCGA database. One hundred sixty-two *NF1* variants were not previously reported in variant databases.

Sequencing of Tumors from Individuals With NF1 Also Demonstrates Osteosarcoma

Tumors from individuals with NF1 included known tumor types, including MPNST, glioma, and gastrointestinal stromal cell tumor,; however, a case of osteosarcoma was also observed. Osteosarcoma in individuals with NF1 has been reported infrequently (Table 1). Furthermore, sequencing of the osteosarcoma tumor demonstrated somatic copy-neutral LOH of *NF1*, in addition to somatic *TP53* and *NOTCH3* variants. The germline splice acceptor *NF1* VAF was 0.48 in the germline and 0.82 in tumor, consistent with biallelic mutation in tumor.

Thirty-eight of the 40 germline NF1 cases had a second hit in the form of deletional loss, copy-neutral LOH, or a somatic point mutation (Table 2 and Fig 1). There were 25 cases that demonstrated LOH of the *NF1* germline in tumor and 13 cases in which an *NF1* germline variant and an *NF1* somatic P/LP alteration were found. The median VAF in normal samples for *NF1* variants was 0.49 ± 0.05, whereas the median VAF in the tumor sample was 0.75 ± 0.19, supporting the importance of LOH in these tumors.

There were four cases with both copy-neutral LOH and an *NF1* somatic hit in the setting of *NF1* germline, but in general, these cases had low VAF for the somatic coding variant with a median VAF of 0.075, likely reflecting tumor heterogeneity/subclonal evolution in these cases.

Comutations seen in these tumors included *CDKN2A/2B* deletion (37.5% of cases), *TP53* mutation (17.5%), and *ATRX* (12.5%). Three cases had *PTEN* pathogenic variants. One case that did not have an identifiable second *NF1* variant had pathogenic variants in *TP53* and *PTEN*. Few instances of pathogenic variants in genes downstream of *NF1* in the RAS signaling pathway (*PIK3CA*, *PTPN11*, *PIK3R1*, and *RAF1*) were noted.

Copy-Neutral LOH is Common in Tumors From Individuals With NF1

Patterns of *NF1* loss were observed including copy-neutral LOH and *NF1* focal deletion/chromosomal loss. 76% of individuals with constitutional *NF1* had tumors that displayed copy-neutral LOH, and 24% had loss/deletion. The results suggested a predominance of copy-neutral LOH in the NF1 cohort here. For tumors that did not have *NF1* germline, 24% of tumors had copy-neutral LOH and 76% had copy loss—deletion (Table 2). Of 351 nongermline-altered *NF1* tumors, 204 had a second hit documented. Small protein-altering NF1 variants represented the second hit in 13 of 38 germline cases, and 96 of 204 nongermline cases had two or more NF1 protein–altering variants without a copy number variant (P = .2, two-proportion z test).

TABLE 1. Reports of Patients With NF1 and Osteosarcoma

Year[citation]	Age, Years	Sex	NF1	Site of Osteosarcoma	Other Tumors	Treatment Notes for Other Tumors
2013[29]	17	Male	+	R distal femur	None	NA
2013[30]	17	Male	+	Femur	MPNST	s/p surgery
2009[31]	37	Male	+	Distal femur	None	NA
2009[31]	21	Male	+	Proximal humerus	None	NA
2009[31]	34	Female	+	Proximal tibia	None	NA
2009[31]	25	Female	+	Distal femur	Spindle cell	s/p chemotherapy, amputation
2006[32]	29	Female	+	Proximal femur	MPNST	s/p chemotherapy, surgery
2002[33]	50	Female	+	Ramus of left mandible	Parathyroid adenoma	NA

Abbreviations: MPNST, malignant peripheral nerve sheath tumor; NA, not available; s/p, status post.

TABLE 2. Patterns of Second Hit in NF1-Altered Tumors

Type of Second Hit	NF1 Germline Cases (n = 38), No. (%)	NF1 Nongermline Cases (n = 204), No. (%)
Copy-neutral LOH[a]	19 (48)	26 (13)
Loss—deletion[b]	6 (15)	82 (40)
Small protein-altering variant[c]	13 (37)	96 (47)

Abbreviations: LOH, loss of heterozygosity; NF1, neurofibromatosis type 1.
[a]Two-sided $P < .0001$, two-proportion z test.
[b]Two-sided $P = .007$, two-proportion z test.
[c]Two-sided $P = .9$, two-proportion z test.

MPNST *NF1*-Altered and Non–*NF1*-Altered

MPNST was also analyzed, and LOH was examined (Fig 2). Eleven MPNSTs were observed; five had constitutional NF1, four did not have *NF1* variants identified, and two were unknown. LOH was commonly observed in MPNST associated with constitutional NF1 (57% *v* 0% in non-NF1 MPNST). *NF1* comutations included *CDKN2A/2B* deletion and *TP53* mutation. Non-*NF1* MPNST had one case with *CDKN2A/2B* deletion and one case with a *TP53* structural rearrangement, in support of the role of these genes in MPNST.[34,35] The PRC2 genes including *EED* and *SUZ12* were sporadically altered, as has been described.[36] Non–*NF1*-related MPNST did not have any recurrent variants. Three of the seven *NF1*-related MPNST cases lacked a second hit *NF1*.

Tumors From Individuals With NF1 Occur at Younger Age

Among *NF1*-altered tumors, we could compare tumors from individuals with constitutional *NF1* germline versus those without. In individuals with germline *NF1*, tumors tended to have fewer pathogenic mutations compared with tumors from individuals who did not have germline *NF1* (3.3 ± 1.4 pathogenic mutations versus 5.3 ± 3.7 pathogenic mutations). The mean age at which tumors were sent for sequencing was 22.7 ± 16.5 years for *NF1* germline cases versus 51.9 ± 22.0 years. The total number of pathogenic mutations was not correlated with age in germline or somatic cases overall ($P = .89$). Ki67 staining correlated with higher-grade tumors, but did not correlate with LOH status.

Second Hit Missense Variants in *NF1* Tumors May Provide Support for Deleteriousness

Although the majority of *NF1* variants are predicted to lead to loss of function, missense variants are more difficult to predict. In the context of an NF1-related tumor with relatively limited pathogenic comutations, somatic missense *NF1* variants may represent a driver event or stochastic coincidence. For tumor missense variants found in combination with an *NF1* germline variant, there were four somatic missense variants in the data. In agreement with ClinVar, G629D had multiple annotations of pathogenicity with a CADD score of 32, suggesting germline and tumor deleteriousness. The variant was seen in combination with a germline splice acceptor variant. Two other missense variants, D2632G and I2615V, were of unclear significance after similar analyses. S82F demonstrates conflicting evidence of pathogenicity in ClinVar although the two recent entries suggest that the variant is pathogenic. The S82F variant was seen in a tumor with a germline *NF1* deletion and had a CADD score of 32. This missense variant with the germline deletion supports the pathogenic role of S82F. Should these variants be found as germline variants, they may similarly confer deleteriousness and be associated with tumor risk.

DISCUSSION

Previous large sequencing efforts with NGS technology of tumor cohorts have revealed germline variants; however, cases of NF1 were rare, with fewer than 10 cases in each of these studies.[37-39] In this study, we present data on tumors

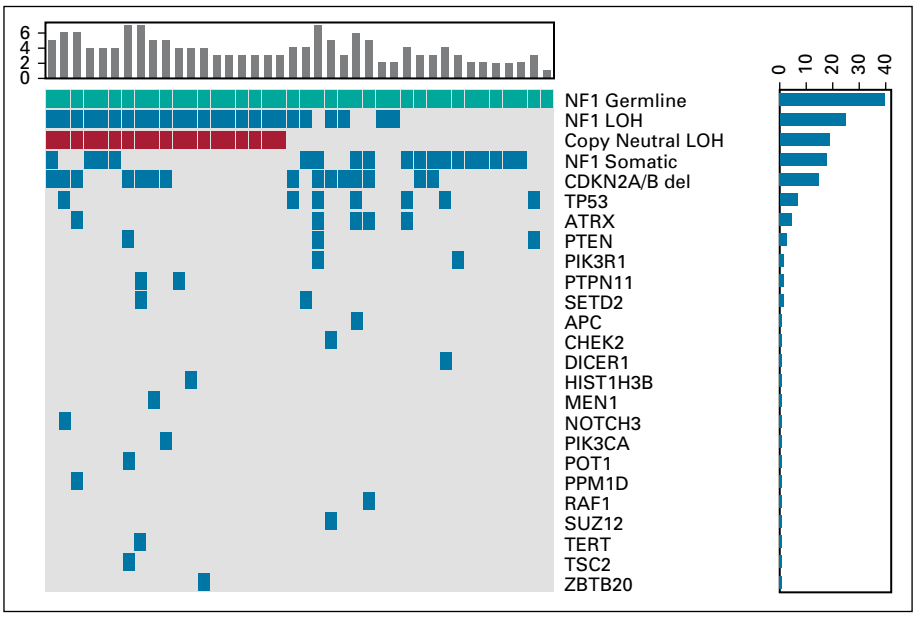

FIG 1. Mutational spectrum of tumors and prevalent copy-neutral LOH of *NF1*. Each box represents a variant correlated with a sample (column). Teal: germline variants. Red: copy-neutral LOH. Blue: tumor variants. NF1 LOH: either deletional loss or copy neutral. CDKN2A/2B del: biallelic deletion. Gray bars in the top: total number of lesions per sample. Blue bars on the right: number of samples that have a given change. LOH, loss of heterozygosity; NF1, neurofibromatosis type 1.

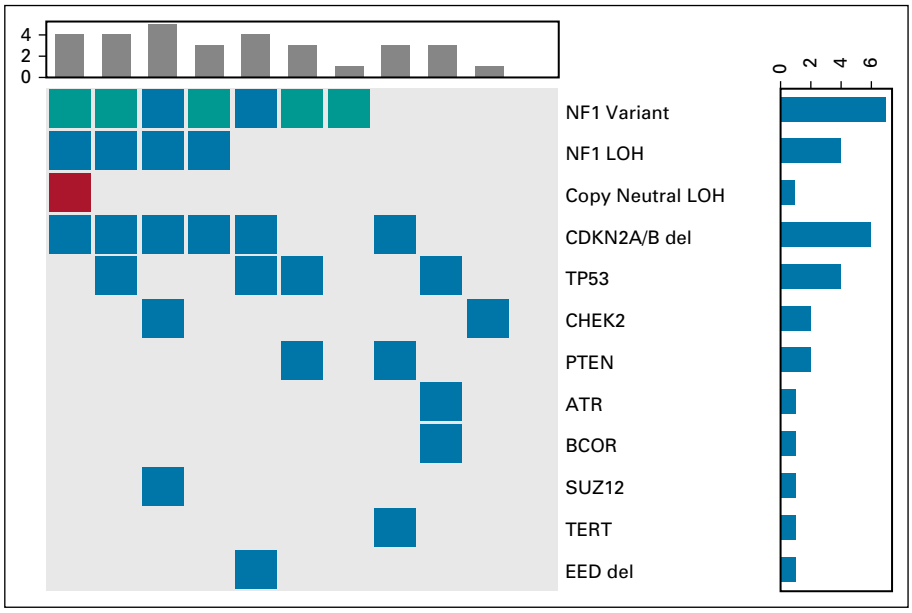

FIG 2. MPNST sequencing and NF1-associated and non–NF1-associated comutations. Each box represents a variant correlated with a sample (column). *NF1* variants: teal corresponds to germline variants, and blue tumor variants. Red: copy-neutral loss of heterozygosity. CDKN2A/2B del: biallelic deletion. EED del: shallow deletion. Gray bars in the top: total number of lesions per sample. Blue bars on the right: number of samples that have a given change. MPNST, malignant peripheral nerve sheath tumor; NF1, neurofibromatosis type 1.

from 40 individuals with NF1 profiled on a multigene sequencing platform. Past sequencing efforts on tumors in NF1 have described specific tumor types, such as atypical neurofibroma (ANF) and MPNST. *CDKN2A/2B* deletion appears to be as a molecular event differentiating ANF and MPNST, and biallelic deletions were more common in MPNST compared with ANF.[36] *TP53* variants have also been noted in MPNST, and in vivo data support a synergistic role for such mutations.[34,35] More recently, the molecular landscape of glioma in individuals with *NF1* has been described and often involves genetic alterations in *TP53*; also, lesions in *CDKN2A/2B* were noted to be prevalent.[40] The cohort of *NF1* tumors in this study further supports the importance of *TP53* and *CDKN2A/2B* in the tumorigenesis.

LOH was prevalent, and our data from tumors in NF1 support that copy-neutral LOH is more common compared with deletion. Copy-neutral LOH is thought to involve mitotic recombination, a process that is known to occur in tumors but that may occur in the general population. Studies investigating the landscape of structural genomic variation have noted that copy-neutral uniparental disomy represented 48% of mosaic events detected in a control population with increasing prevalence with age and most common on chromosomes 9 and 14.[41-43] 80% of the events were telomeric on the p or q arm. Only three events were detected in *NF1*. In studies of NF1, mitotic recombination in *NF1* tended to be centromeric; although copy-neutral LOH is likely seen in the general population, the specific location does not seem to mirror the profile in patients with NF1. The mechanism driving copy-neutral LOH has not been fully elucidated. Studies have suggested that BRCA2, DNA-protein kinase, and POLQ may suppress interhomolog recombination although these findings were not specific to copy-neutral LOH at the *NF1* locus.[44] Biallelic inactivation of homologous recombination repair genes has further been linked to genome-wide LOH.[45] Copy-neutral LOH has also been documented in tumor suppressors including *TSC1/2*, *BRCA1/2*, and *TP53* in other tumors as well, emphasizing this mechanism in tumorigenesis.[46,47]

The tumor subtypes represented tumors commonly associated with NF1. Rare tumors such as osteosarcoma in NF1 are of interest as osteosarcoma has been reported in NF1 and can be challenging to treat.[29-33] Of eight cases of osteosarcoma, two were postsurgery and chemotherapy at a site of a previous malignancy. None of the literature cases had genomic characterization of tumors to our knowledge. The DKFZ Pediatric Pan-Cancer collection of genomic characterization has 42 cases of osteosarcoma, but none of these have *NF1* alteration. One study estimated an increased prevalence of osteosarcoma in individuals with NF1 compared with control; however, further data are needed to make further estimates.[3] Therefore, the molecular data presented here may represent a unique report of NGS from osteosarcoma in NF1 with documented comparison of tumors with constitutional sequencing. The germline *NF1* splice variant demonstrated copy-neutral LOH in tumor with a corresponding elevated VAF. In addition, the tumor demonstrated somatic *NOTCH3* and *TP53* pathogenic variants in addition to the LOH of *NF1*, suggesting that these genes may be important in this setting. Although *TP53* variants are common in osteosarcoma, *NOTCH3* variants are less common.[48,49] Further sequencing studies will be needed to further examine the tumor profiles and association.

Among cases of MPNST, two subcategories are evident: those with *NF1* alteration and those without. *TP53, EED, SUZ12,* and *CDKN2A/2B* deletions were seen in the data set. Furthermore, among *NF1*-altered MPNST, not all cases demonstrated a second hit in *NF1*. Compared with TCGA

data, this study has a similar number of *NF1*-altered MPNST cases (8 of 11 *v* 6 of 14 on TCGA). In the TCGA data set, 3 of 6 demonstrated two *NF1* lesions. Previous analysis of germline and somatic variations in *NF1* in MPNST revealed large deletions including the *NF1* locus in 91% of their cohort. Their data set also had 2 cases with NF1 germline without evidence of a second hit,[50] and early studies had identified *TP53* loss in some cases that lacked a second *NF1* hit.[51] Although MPNST is a key risk lesion for patients with NF1, a second hit in *NF1* is not always identified.

NGS may also contribute to the interpretation of *NF1* variant deleteriousness. For example, missense variants can be challenging to annotate (eg, ClinVar has 293 conflicting and 3,106 VUS in *NF1*). The study here evaluated *NF1* variants that occurred somatically as second hits in the context of germline *NF1* variants. Could this information potentially be applied to the variant's germline variant classification criteria? Current American College of Medical Genetics and Genomics classification allows for determination of pathogenicity of variants on the basis of functional studies.[52] The increasing information from genomic studies of paired tumor and normal samples may represent an avenue of assessment of variants given a proper context, that is, if a change can be considered a potential driver and phenomena such as hypermutation are absent.

At this time, tumor-only testing is more common compared with paired germline and tumor testing, in part due to costs and insurance. Tumor-only testing has advantages but has limitations in terms of inferring germline status from VAF.[53] The data presented here highlight another potential benefit of paired testing: improving variant interpretation of not only somatic but also corresponding germline variation.

This study has a number of limitations. As data are from a tertiary center, the cohort may be enriched for specific types of tumors or cases that may harbor certain types of genetic changes. Brain tumors represent the most common tumors in the data set, which could also affect the variants observed. Although the platform has a sequencing depth of $> 500\times$, it is largely limited to coding regions, and therefore, intron or promoter variants that could affect the gene are not specifically detected. The resolution of copy number calling is also limited by probe density. *NF1* variants were also unphased, which is a limitation to interpretation of small variants, eg, whether these are in cis or trans. Age in our study was time of panel sequencing, which may be delayed relative to surgical resection or diagnosis.

The results add to the data on *NF1*-altered tumors, particularly tumors that arise in the context of individuals with NF1. The sample size and analysis of LOH emphasize the importance of copy-neutral heterozygosity in these tumors. Osteosarcoma, a tumor type not traditionally thought to be NF1-related, appears to be mechanistically related to double hits in NF1. This tumor in addition to others reported in the cohort here shows a spectrum of second alterations in the context of *NF1* germline that favor copy-neutral LOH rather than deletion in lesions with limited genetic variation. Future studies on paired normal tissue and tumor may be able to provide further guidance on mechanisms to target in potential therapeutic strategies.

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DATA SHARING STATEMENT

All data are available upon request. Further methods are also available by contacting the laboratory.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Evans DG, Howard E, Giblin C, et al: Birth incidence and prevalence of tumor-prone syndromes: Estimates from a UK family genetic register service. Am J Med Genet A 152A:327-332, 2010
2. Korf BR: Malignancy in neurofibromatosis type 1. Oncologist 5:477-485, 2000
3. Landry JP, Schertz KL, Chiang Y-J, et al: Comparison of cancer prevalence in patients with neurofibromatosis type 1 at an Academic Cancer Center vs in the general population from 1985 to 2020. JAMA Netw Open 4:e210945, 2021
4. Miettinen MM, Antonescu CR, Fletcher CDM, et al: Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in neurofibromatosis 1 patients—A consensus overview. Hum Pathol 67:1-10, 2017
5. Miller DT, Freedenberg D, Schorry E, et al: Health supervision for children with neurofibromatosis type 1. Pediatrics 143:e20190660, 2019
6. Stewart DR, Korf BR, Nathanson KL, et al: Care of adults with neurofibromatosis type 1: A clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). Genet Med 20:671-682, 2018
7. Pemov A, Li H, Patidar R, et al: The primacy of NF1 loss as the driver of tumorigenesis in neurofibromatosis type 1-associated plexiform neurofibromas. Oncogene 36:3168-3177, 2017
8. Serra E, Rosenbaum T, Nadal M, et al: Mitotic recombination effects homozygosity for NF1 germline mutations in neurofibromas. Nat Genet 28:294-296, 2001
9. Garcia-Linares C, Fernández-Rodríguez J, Terribas E, et al: Dissecting loss of heterozygosity (LOH) in neurofibromatosis type 1-associated neurofibromas: Importance of copy neutral LOH. Hum Mutat 32:78-90, 2011
10. Margraf RL, VanSant-Webb C, Mao R, et al: NF1 somatic mutation in dystrophic scoliosis. J Mol Neurosci 68:11-18, 2019
11. Stewart DR, Pemov A, Van Loo P, et al: Mitotic recombination of chromosome arm 17q as a cause of loss of heterozygosity of NF1 in neurofibromatosis type 1-associated glomus tumors. Genes Chromosomes Cancer 51:429-437, 2012
12. Stevenson DA, Zhou H, Ashrafi S, et al: Double inactivation of NF1 in tibial pseudarthrosis. Am J Hum Genet 79:143-148, 2006
13. Steinemann D, Arning L, Praulich I, et al: Mitotic recombination and compound-heterozygous mutations are predominant NF1-inactivating mechanisms in children with juvenile myelomonocytic leukemia and neurofibromatosis type 1. Haematologica 95:320-323, 2010
14. Gutmann DH, McLellan MD, Hussain I, et al: Somatic neurofibromatosis type 1 (NF1) inactivation characterizes NF1-associated pilocytic astrocytoma. Genome Res 23:431-439, 2013
15. Upadhyaya M, Han S, Consoli C, et al: Characterization of the somatic mutational spectrum of the neurofibromatosis type 1 (NF1) gene in neurofibromatosis patients with benign and malignant tumors. Hum Mutat 23:134-146, 2004
16. Parsons DW, Roy A, Yang Y, et al: Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. JAMA Oncol 2:616-624, 2016
17. Hicks JK, Howard R, Reisman P, et al: Integrating somatic and germline next-generation sequencing into routine clinical oncology practice. JCO Precis Oncol 5:884-895, 2021
18. Dumbrava EI, Brusco L, Daniels M, et al: Expanded analysis of secondary germline findings from matched tumor/normal sequencing identifies additional clinically significant mutations. JCO Precis Oncol 3:1-11, 2019
19. Ritter DI, Rao S, Kulkarni S, et al: A case for expert curation: An overview of cancer curation in the Clinical Genome Resource (ClinGen). Cold Spring Harb Mol Case Stud 5:a004739, 2019
20. Tate JG, Bamford S, Jubb HC, et al: COSMIC: The Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res 47:D941-D947, 2019
21. Landrum MJ, Lee JM, Benson M, et al: ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Res 46:D1062-D1067, 2018
22. Karczewski KJ, Francioli LC, Tiao G, et al: The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581:434-443, 2020
23. Gao J, Aksoy BA, Dogrusoz U, et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6:pl1, 2013
24. Cerami E, Gao J, Dogrusoz U, et al: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 2:401-404, 2012
25. Lee W, Teckie S, Wiesner T, et al: PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat Genet 46:1227-1232, 2014
26. Mikhail FM, Biegel JA, Cooley LD, et al: Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: A joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). Genet Med 21:1903-1916, 2019
27. Gu Z, Eils R, Schlesner M: Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32:2847-2849, 2016
28. R Core Team: R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing, 2017. https://www.R-project.org/
29. Cheuk DK, Chiang AK, Ha SY, et al: Malignancies in Chinese patients with neurofibromatosis type 1. Hong Kong Med J 19:42-49, 2013
30. Afşar ÇU, Kara İO, Kozat BK, et al: Neurofibromatosis type 1, gastrointestinal stromal tumor, leiomyosarcoma and osteosarcoma: Four cases of rare tumors and a review of the literature. Crit Rev Oncol Hematol 86:191-199, 2013
31. Chowdhry M, Hughes C, Grimer RJ, et al: Bone sarcomas arising in patients with neurofibromatosis type 1. J Bone Joint Surg Br 91-B:1223-1226, 2009
32. Hatori M, Hosaka M, Watanabe M, et al: Osteosarcoma in a patient with neurofibromatosis type 1: A case report and review of the literature. Tohoku J Exp Med 208:343-348, 2006
33. Cinamon U, Avinoach I, Harell M: Neurofibromatosis type 1, hyperparathyroidism, and osteosarcoma: Interplay? Eur Arch Otorhinolaryngol 259:540-542, 2002
34. Vogel KS, Klesse LJ, Velasco-Miguel S, et al: Mouse tumor model for neurofibromatosis type 1. Science 286:2176-2179, 1999
35. Legius E, Dierick H, Wu R, et al: TP53 mutations are frequent in malignant NF1 tumors. Genes Chromosomes Cancer 10:250-255, 1994
36. Pemov A, Hansen NF, Sindiri S, et al: Low mutation burden and frequent loss of CDKN2A/B and SMARCA2, but not PRC2, define premalignant neurofibromatosis type 1–associated atypical neurofibromas. Neuro Oncol 21:981-992, 2019
37. Fiala EM, Jayakumaran G, Mauguen A, et al: Prospective pan-cancer germline testing using MSK-IMPACT informs clinical translation in 751 patients with pediatric solid tumors. Nat Cancer 2:357-365, 2021
38. Gröbner SN, Worst BC, Weischenfeldt J, et al: The landscape of genomic alterations across childhood cancers. Nature 555:321-327, 2018
39. Lincoln SE, Nussbaum RL, Kurian AW, et al: Yield and utility of germline testing following tumor sequencing in patients with cancer. JAMA Netw Open 3:e2019452, 2020
40. D'Angelo F, Ceccarelli M, Tala, et al: The molecular landscape of glioma in patients with Neurofibromatosis 1. Nat Med 25:176-187, 2019
41. Machiela MJ, Zhou W, Sampson JN, et al: Characterization of large structural genetic mosaicism in human autosomes. Am J Hum Genet 96:487-497, 2015

42. Jacobs KB, Yeager M, Zhou W, et al: Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet 44:651-658, 2012
43. Laurie CC, Laurie CA, Rice K, et al: Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet 44:642-650, 2012
44. Davis L, Khoo KJ, Zhang Y, et al: POLQ suppresses interhomolog recombination and loss of heterozygosity at targeted DNA breaks. Proc Natl Acad Sci USA 117:22900-22909, 2020
45. Westphalen B, Fine AD, Andre F, et al: Pan-cancer analysis of homologous recombination repair-associated gene alterations and genome-wide loss of heterozygosity score. Clin Cancer Res 28:1412-1421, 2021
46. Giannikou K, Malinowska IA, Pugh TJ, et al: Whole exome sequencing identifies TSC1/TSC2 biallelic loss as the primary and sufficient driver event for renal angiomyolipoma development. PLoS Genet 12:e1006242, 2016
47. Zhang X, Sjöblom T: Targeting loss of heterozygosity: A novel paradigm for cancer therapy. Pharmaceuticals (Basel) 14:57, 2021
48. Bishop MW, Janeway KA, Gorlick R: Future directions in the treatment of osteosarcoma. Curr Opin Pediatr 28:26-33, 2016
49. Roberts RD, Lizardo MM, Reed DR, et al: Provocative questions in osteosarcoma basic and translational biology: A report from the Children's Oncology Group. Cancer 125:3514-3525, 2019
50. Upadhyaya M, Kluwe L, Spurlock G, et al: Germline and somatic NF1 gene mutation spectrum in NF1-associated malignant peripheral nerve sheath tumors (MPNSTs). Hum Mutat 29:74-82, 2008
51. Menon AG, Anderson KM, Riccardi VM, et al: Chromosome 17p deletions and p53 gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis. Proc Natl Acad Sci USA 87:5435-5439, 1990
52. Li MM, Chao E, Esplin ED, et al: Points to consider for reporting of germline variation in patients undergoing tumor testing: A statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med 22:1142-1148, 2020
53. Jalloul N, Gomy I, Stokes S, et al: Germline testing data validate Inferences of mutational status for variants detected from tumor-only sequencing. JCO Precis Oncol 5:1749-1757, 2021

APPENDIX

TABLE A1. Distribution of Cancer Types Among UCSF500 Samples

Anatomic Category	No. of Tumors (n = 6,168)	Tumors With NF1 Pathogenic or Likely Pathogenic Alteration (n = 391)
CNS	2,152	226
Skin	379	39
Unknown primary	585	22
Lung	342	17
Uterus	142	15
Peripheral nerve	81	14
Others	771	13
Ovarian/fallopian	172	13
Soft tissue	154	8
Bladder/urinary tract	120	5
Breast	119	4
Head and neck	58	3
Lymphoid/myeloid	181	3
Bowel	86	2
Thyroid	91	2
Ampulla of vater	6	1
Bone	47	1
Pancreas	82	1
Stomach	5	1
Stomach/esophagus	52	1
Adrenal	11	0
Appendix	5	0
Biliary	26	0
Cervix	10	0
Eye	46	0
Germ	7	0
Kidney	90	0
Liver	24	0
Pleura	6	0
Prostate	79	0
Thymus	3	0
Unknown	234	0
Vagina	2	0

Abbreviation: NF1, neurofibromatosis type 1.

TABLE A2. Spectrum of *NF1*-Altered Tumors

Pathologic Category	*NF1*-Altered Tumors (n = 391)
Glioblastoma	79
Glioma	72
Astrocytoma	40
Melanoma	33
Others	26
Unknown primary	20
Lung adenocarcinoma	15
Gliosarcoma	10
Uterine cancer	9
Diffuse intrinsic pontine glioma	8
Malignant peripheral nerve sheath tumor	7
Ovarian cancer	7
Neurofibroma	5
Breast cancer	4
Bladder urothelial carcinoma	4
Sarcoma	3
Rosette-forming glioneuronal tumor of the fourth ventricle	3
Spindle cell neoplasm	3
Head and neck squamous cell carcinoma	2
Ganglioglioma	2
Endometrial carcinoma	2
Dysembryoplastic neuroepithelial tumor	2
Colorectal cancer	2
Atypical nevus	2
Adenocarcinoma, NOS	2
Urachal adenocarcinoma	1
Subependymoma	1
Poorly differentiated thyroid cancer	1
Plexiform neurofibroma	1
Pleomorphic xanthoastrocytoma	1
Pheochromocytoma	1
Ovarian epithelial tumor	1
Osteosarcoma	1
Oligodendroglioma	1
Ocular melanoma	1
Neuroepithelial tumor	1
Myeloproliferative neoplasms	1
Mucosal melanoma of the urethra	1
Mixed phenotype acute leukemia, T/myeloid, NOS	1
Miscellaneous neuroepithelial tumor	1
Malignant neurocristic neoplasm	1
Lung squamous cell carcinoma	1
Large cell neuroendocrine carcinoma	1
High-grade serous fallopian tube cancer	1
GIST	1
Embryonal rhabdomyosarcoma	1
B-cell lymphoma	1
B-cell acute lymphoid leukemia	1
Atypical teratoid/rhabdoid tumor	1
Anaplastic thyroid cancer	1
Anaplastic oligodendroglioma	1
Anaplastic meningioma	1
Anaplastic ependymoma	1
Ampullary carcinoma	1

Abbreviations: GIST, gastrointestinal stromal cell tumor; NOS, not otherwise specified.