Neurofibromatosis-1 gene mutational profiles differ between syndromic disease and sporadic cancers

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Objectives
Variants in the neurofibromatosis type 1 (NF1) gene are not only responsible for the NF1 cancer predisposition syndrome, but also frequently identified in cancers arising in the general population. While germline variants are pathogenic, it is not known whether those that arise in cancer (somatic variants) are passenger or driver variants. To address this question, we sought to define the landscape of NF1 variants in sporadic cancers.

Methods
NF1 variants in sporadic cancers were compiled using data curated on the c-Bio database and compared with published germline variants and Genome Aggregation Database data. Pathogenicity was determined using Polyphen and Sorting Intolerant From Tolerant prediction tools.

Results
The spectrum of NF1 variants in sporadic tumors differ from those most commonly seen in individuals with NF1. In addition, the type and location of the variants in sporadic cancer differ from germline variants, where a high proportion of missense variants were found. Finally, many of the sporadic cancer NF1 variants were not predicted to be pathogenic.

Discussion
Taken together, these findings suggest that a significant proportion of NF1 variants in sporadic cancer may be passenger variants or hypomorphic alleles. Further mechanistic studies are warranted to define their unique roles in nonsyndromic cancer pathobiology.
Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome affecting 1 in 3,000 individuals worldwide (OMIM: 162200). While individuals with NF1 frequently present with pigmented abnormalities, there is also a higher incidence of both benign and malignant tumors, including peripheral nerve sheath tumors, gliomas, pheochromocytoma, and breast cancer. In addition, the NF1 gene is one of the most frequently mutated genes in cancers of the general population, with variant frequencies ranging from 15% to 70%.1 While germline variants in individuals with NF1 are assuredly disease causing, it is unclear whether somatic variants identified in the setting of sporadic cancer represent pathogenic variants important for neoplastic progression or passenger variants with little effect on oncogenesis. Herein, we compared the NF1 variant spectrum in patients with NF1 (germline) with those detected in sporadic cancers.

Methods

Somatic NF1 variants in sporadic cancers were assembled from cBioPortal.2 Duplicate samples, defined as having the same sample identification number and variant, were eliminated. Cancer types harboring fewer than 13 NF1 variants were excluded. NF1 gene variant type and location were compared with all published germline NF1 variants from patients known to have NF1 based on clinical diagnostic criteria3,4 and Genome Aggregation Database (gnomAD) data.5 To evaluate variant location, the neurofibromin protein was divided into tertiles, representing amino acids 1–939, 940–1878, and 1879–2818.3 Pathogenicity was determined using Polyphen and Sorting Intolerant From Tolerant. Two-sample t tests were used to compare the percentage of germline and sporadic variants in each neurofibromin tertile. The Fisher exact test was
used to compare the frequency of germline and sporadic variant types. Statistical significance was set at $p < 0.05$. R Script adapted from Plot Protein was used to map variants onto the NF1 isoform P21359-2 (NP_000258.1). Additional data and references are provided in eTables 1 and 2 and eReferences (links.lww.com/NXG/A533).

**Data Availability**

Data were deidentified and available in publicly accessible databases or from published reports, not requiring institutional review board approval or new data deposition.

**Results**

Thirty-eight different sporadic cancers, harboring 2,176 somatic NF1 variants, were compared with 1,161 germline variants, including 298 NF1 variants from patients with known NF1 and 863 germline polymorphic variants from gnomAD. First, we found that cancer types with the highest frequency of NF1 variants were not those commonly overrepresented in people with NF1 (Figure 1). As such, melanoma and uterine carcinoma had the highest percentages of NF1 variants while those cancers seen in individuals with NF1 ranged from <1% (breast cancer) to 21% (glioblastoma). Second, although there were fewer NF1 variants in the third tertile of the NF1 gene in individuals with NF1 ($p = 0.047$), as previously reported in children (12%) and adults with other NF1-associated brain tumors (11%) (Figure 2A), NF1 variants were evenly distributed along the NF1 coding sequence in sporadic cancers (Figure 2B). Similarly, in previous studies examining NF1-associated brain tumors (low-grade and high-grade gliomas), somatic variants were also spread broadly throughout the NF1 gene, while the germline variants were found to have a slight bias toward the 5’ end of the gene and did not cluster in specific domain regions. Using synonymous variants as a control, there were no significant differences in the distribution of germline and sporadic variants (Figure 2, A and B). Third, while NF1 germline variants were mainly nonsense and frameshift variants (61%), sporadic cancer NF1 variants were mostly missense variants (48%) (Figure 2C). A visual depiction of the location and frequency of the sporadic and germline missense variants shows this difference in NF1 variant profiles (Figure 3A). Despite comprising half of all sporadic cancer variants, most missense NF1 variants were not predicted to be pathogenic, with only 1.4%

**Figure 2** Comparison of Variant Distribution and Type in Patients With NF1 vs Sporadic Cancers

(A) The percentage of missense and loss-of-function NF1 variants found in each tertile of the NF1 protein (neurofibromin) for individuals with NF1 (white bars) relative to sporadic cancers (gray bars). Error bars represent standard errors of the mean (2-sample t-test). The germline variants include data from 3 sources of NF1 germline variants. Individual points represent variants from each curated list of germline variants or sporadic cancer type. (B) The percentage of germline (white bars) and somatic (gray bars) synonymous variants located in each tertile of the NF1 protein (Fisher exact test). (C) The percentage of each type of NF1 variant, grouped into germline (white bars) and somatic (gray bars) pairs (Fisher exact test). NF1 = neurofibromatosis type 1.
of all somatic variants estimated to be pathogenic. In addition, only 3% of all sporadic cancer NF1 missense variants were predicted to be pathogenic. Further analysis revealed that 16 missense variants occurred 4 or more times (Figure 3B), where 11 (69%) were predicted to be pathogenic.

**Discussion**

Taken together, these results demonstrate that NF1 variants in sporadic cancers differ both in location and type relative to germline variants from individuals with NF1, and that cancers with the largest frequency of NF1 variants were not the tumors most prevalent in the setting of NF1 clinical disease. Of interest, the preponderance of missense variants in sporadic cancers raises the intriguing possibility that some of these NF1 variants, especially missense variants, could represent nonpathogenic “passenger”, or hypomorphic, variants. However, a majority of recurrent missense mutations were predicted to be pathogenic. Future studies that aim to define the effects of these variants on neurofibromin structure, protein interactions, and function are required to determine their significance to oncogenesis.
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Disclosure
The authors report no disclosures. Go to Neurology.org/NG for full disclosures.

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References

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