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Neurofibromatosis-1 Gene Mutational Profiles Differ Between Syndromic Disease and Sporadic Cancers

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Abstract

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.

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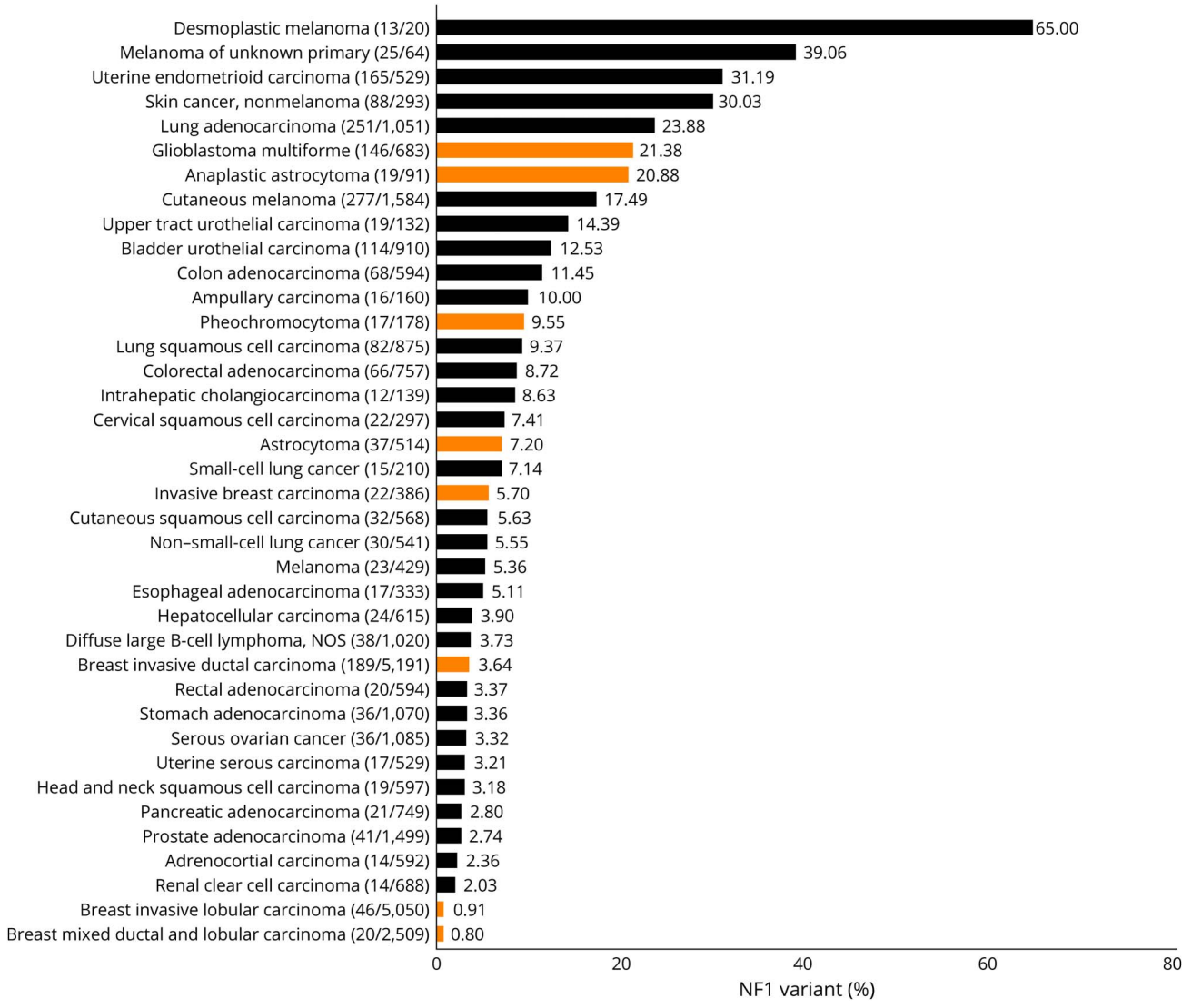
Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome affecting 1 in 3,000 individuals worldwide (OMIM: 162200). While individuals with NF1 frequently present with pigmentary abnormalities, there is also a higher incidence of both benign and malignant tumors, including peripheral nerve sheath 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 criteria[3,4] 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

Figure 1 Percentage of *NF1* Variants in Sporadic Cancers



Bar chart depicting the percentage of *NF1* variants found in each cancer type. The percentages were calculated by dividing the number of samples harboring an *NF1* variant by the total number of cancer samples in each cancer type. The orange bars denote those cancers more prevalent in individuals with NF1. NF1 = neurofibromatosis type 1.

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).[6] Additional data and references are provided in eTables 1 and 2 and eReferences (links.lww.com/NXG/A533).

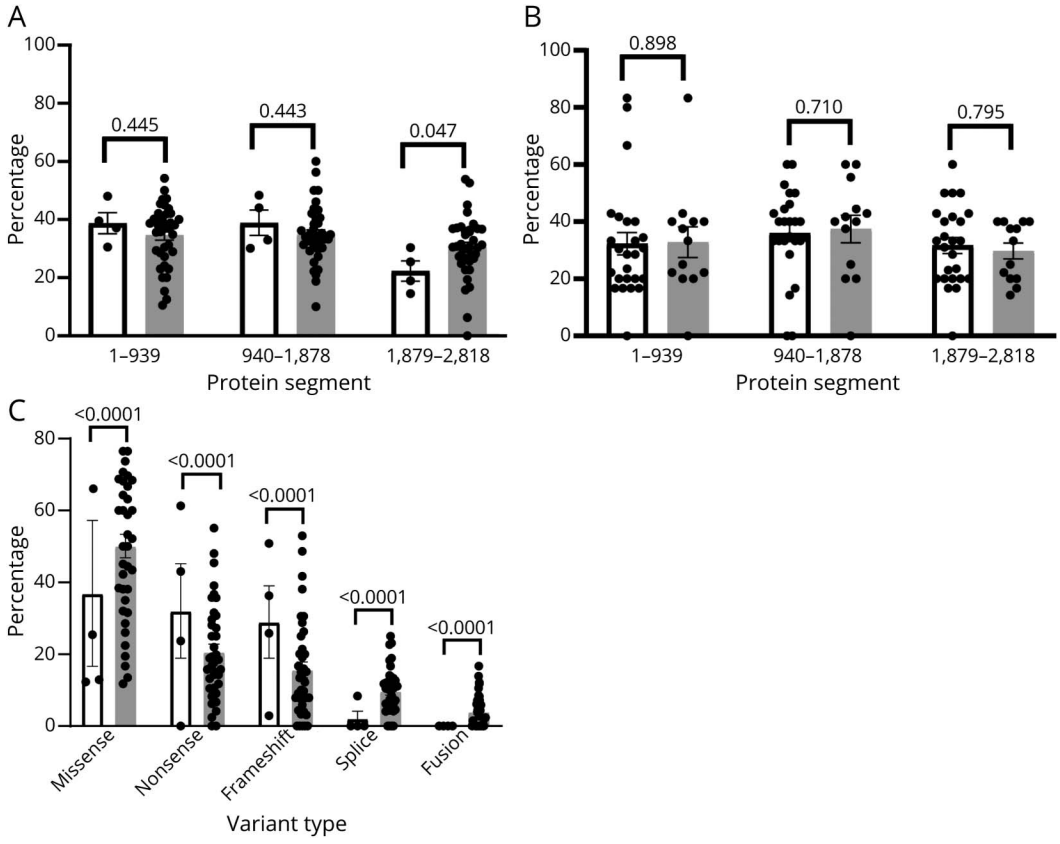
Data Availability

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

Results

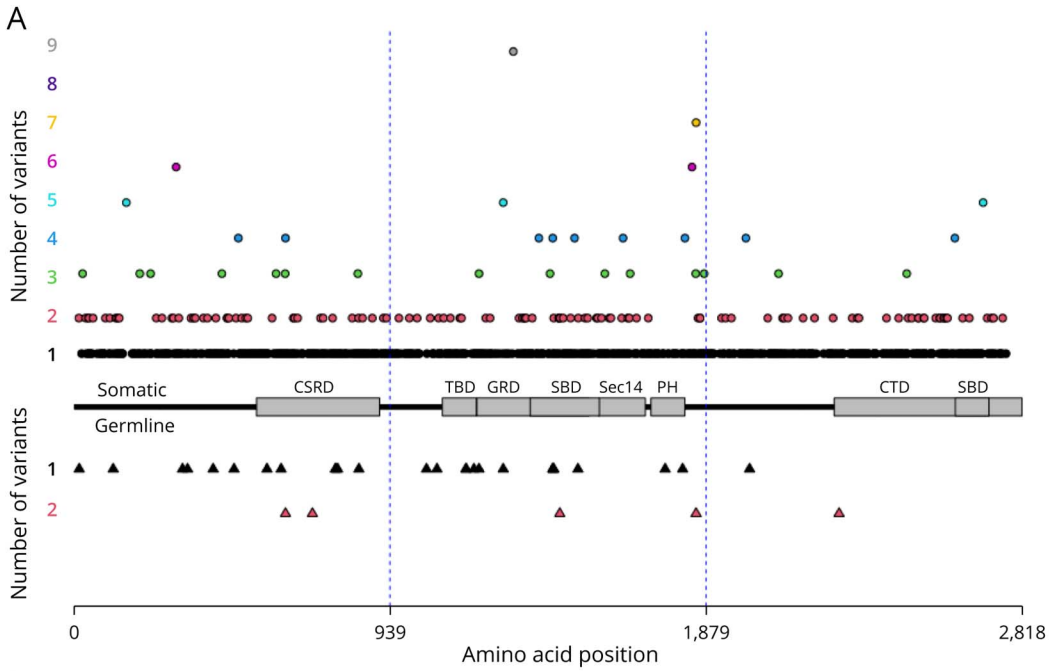
Thirty-eight different sporadic cancers, harboring 2,176 somatic *NF1* variants, were compared with 1,161 germline variants, including 298 *NF1* variants from patients with known NF1 and 863 germline polymorphic variants from gnomAD. First, we found that cancer types with the highest frequency of *NF1* 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%)[3] and adults with other NF1-associated brain tumors (11%)[7] (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.[6,7] 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.[3-5] 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.

Figure 3 Distribution and Pathogenicity of Missense *NF1* Variants



B

Variant	N	Allele freqeuncy	Pathogenicity		gnomAD
			Polyphen	SIFT	
R1306Q	9	3.977×10^{-6}	Benign	Tolerated	Yes
R1870Q	7		Probably damaging	Deleterious	No
R304Q	6		Possibly damaging	Deleterious	No
A1858T	6	3.979×10^{-6}	Probably damaging	Deleterious	Yes
R156H	5	3.981×10^{-6}	Probably damaging	Deleterious	Yes
R1276Q	5	3.981×10^{-6}	Probably damaging	Deleterious	Yes
Q2723R	5		Benign	Tolerated	No
Y489C	4	1.197×10^{-5}	Benign	Tolerated	Yes
G629R	4	3.981×10^{-6}	Benign	Tolerated	Yes
G1403S	4	4.378×10^{-5}	Possibly damaging	Tolerated	Yes
K1444N	4		Probably damaging	Deleterious	No
R1509C	4		Probably damaging	Deleterious	No
R1653H	4	1.989×10^{-5}	Probably damaging	Deleterious	Yes
D1837N	4	7.959×10^{-6}	Benign	Tolerated	Yes
S2018I	4		Probably damaging	Deleterious	No
L2639I	4		Probably damaging	Deleterious	No

(A) Visual representation of missense *NF1* variants in sporadic cancers (circles; somatic variants) and NF1 patients (triangles; germline variants), with the numbers and locations arrayed along the *NF1* protein (neurofibromin) coding sequence. Color corresponds to the number of times each variant occurred (y-axis number colors). Vertical lines indicate the boundaries of the neurofibromin tertiles. Known structural motifs are depicted including the CSRD, cysteine-serine-rich domain; TBD, tubulin binding domain; GRD, GTPase-activating protein-related domain; SBD, syndecan binding domain; Sec14, Sec14 domain; PH, pleckstrin homology domain; CTD, carboxy-terminal domain. (B) Recurrent missense variants (N) in sporadic cancers are listed with their allele frequencies, predicted pathogenicity (using Polyphen and SIFT), and presence in gnomAD. gnomAD = Genome Aggregation Database; NF1 = neurofibromatosis type 1l SIFT = Sorting Intolerant From Tolerant.

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

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Alice F. Bewley, MS	Washington University School of Medicine, St. Louis, MO	Performed the analyses, prepared the figures, and wrote the manuscript drafts
Titilope M. Akinwe, BS	Washington University School of Medicine, St. Louis, MO	Prepared Figure 3A and performed clustering analyses
Tychele N. Turner, PhD	Washington University School of Medicine, St. Louis, MO	Edited the manuscript
David H. Gutmann, MD, PhD	Washington University School of Medicine, St. Louis, MO	Designed the study and edited the manuscript, corresponding author

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