



Case report

Clonal lineage of high grade serous ovarian cancer in a patient with neurofibromatosis type 1

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ABSTRACT

Neurofibromatosis type 1 (NF1) is caused by mutations in the *NF1* gene encoding neurofibromin, which negatively regulates Ras signaling. NF1 patients have an increased risk of developing early onset breast cancer, however, the association between NF1 and high grade serous ovarian cancer (HGSOC) is unclear. Since most NF1-related tumors exhibit early biallelic inactivation of *NF1*, we evaluated the evolution of genetic alterations in HGSOC in an NF1 patient. Somatic variation analysis of whole exome sequencing of tumor samples from both ovaries and a peritoneal metastasis showed a clonal lineage originating from an ancestral clone within the left adnexa, which exhibited copy number (CN) loss of heterozygosity (LOH) in the region of chromosome 17 containing *TP53*, *NF1*, and *BRCA1* and mutation of the other *TP53* allele. This event led to biallelic inactivation of *NF1* and *TP53* and LOH for the *BRCA1* germline mutation. Subsequent CN alterations were found in the dominant tumor clone in the left ovary and nearly 100% of tumor at other sites. Neurofibromin modeling studies suggested that the germline *NF1* mutation could potentially alter protein function. These results demonstrate early, biallelic inactivation of neurofibromin in HGSOC and highlight the potential of targeting RAS signaling in NF1 patients.

1. Introduction

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders affecting approximately 1 in 3500 individuals. The disease is caused by mutations in the *NF1* gene and shows complete penetrance (Yap et al., 2014). *NF1*, which encodes a GTPase-activating protein (neurofibromin) that negatively regulates RAS-signaling pathways, is considered a classical tumor suppressor gene. NF1 patients have an estimated lifetime risk of 59.6% of developing cancer resulting in a decreased life expectancy of 10–15 years (Walker et al., 2006). While *NF1* haploinsufficiency may have functional consequences, biallelic inactivation of *NF1* frequently precedes or occurs simultaneously with malignant transformation in NF1-related cancers (Yap et al., 2014; Maertens et al., 2006).

Recent evidence suggests that NF1 is associated with an increased incidence of early onset breast cancer and biallelic inactivation of *NF1*

is an early event in tumorigenesis (McPherson et al., 2015). An association between NF1 and ovarian cancer is emerging and somatic mutations and copy number alterations (CNA) of *NF1* are frequently observed in high grade serous ovarian cancer (HGSOC) (Cancer Genome Atlas Research N, 2011; Patil and Chamberlain, 2012; Kanchi et al., 2014; Salud et al., 1991; Ceccaroni et al., 2002; Jeon et al., 2015). We present the case of a woman who developed two separate NF1-related malignancies (malignant peripheral nerve sheath tumor (MPNST) and HGSOC) before the age of 44. Whole exome sequencing (WES) of tumor DNA from bilateral ovaries and peritoneal metastasis was performed to investigate tumor evolution and determine whether biallelic inactivation of *NF1* is an early event of ovarian carcinogenesis.

2. Case history

A 44-year-old gravida 5, para 3 African American female presented

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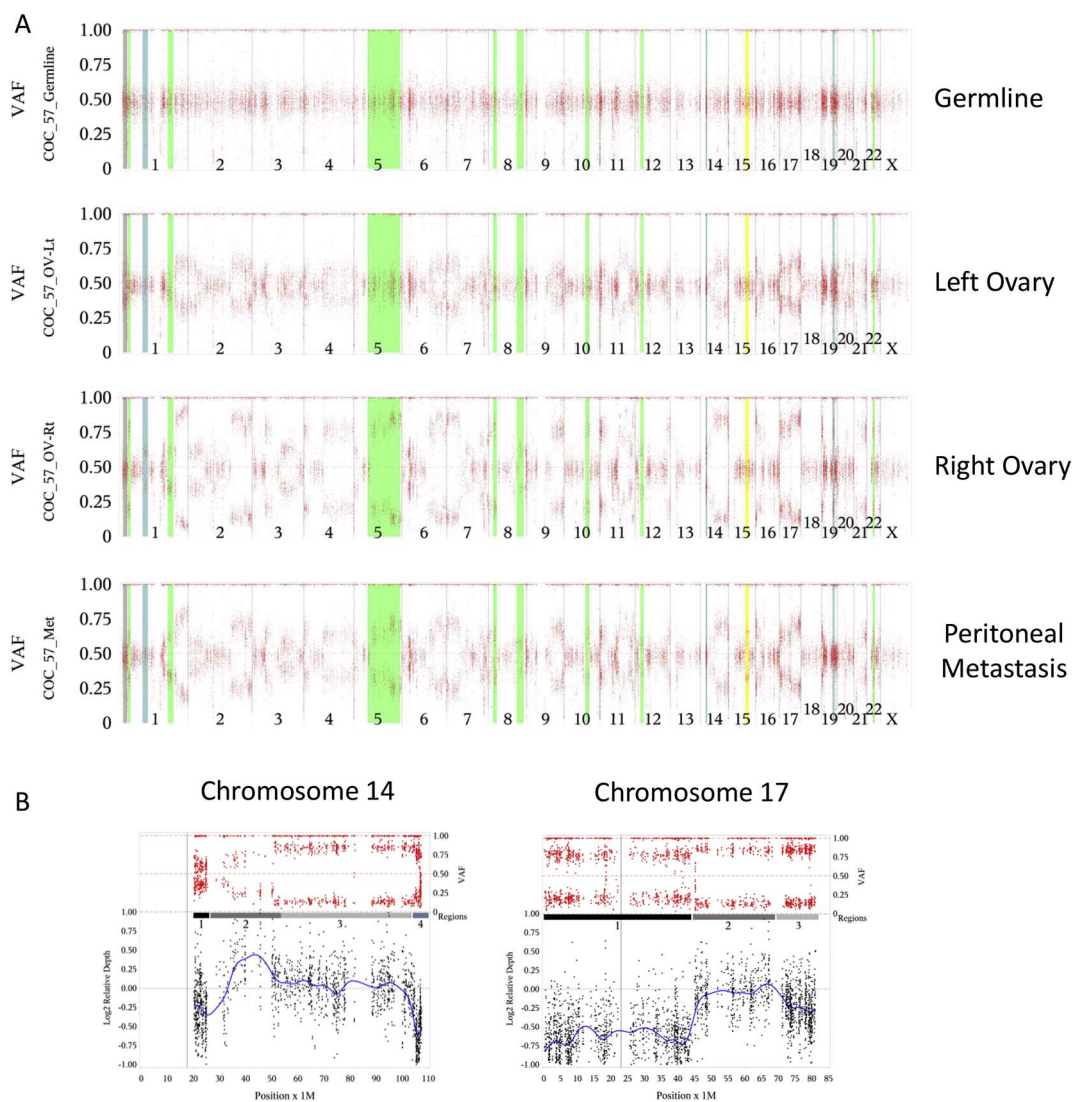


Fig. 1. Copy Number Alterations. (A) Genomic profile of CNA in tumor samples isolated from the left ovary, right ovary, and peritoneal metastasis. Germline DNA was used as a reference. Regions of interest are shaded as unique to left ovary (grey), unique to right ovary (blue), unique to peritoneal metastasis (yellow), and common between right ovary and peritoneal metastasis (green). (B) A detailed Variant Allele Frequency and Relative Depth Log Ratio plot from the right ovary tumor sample indicating the regions where copy number alterations occurred on chromosomes 14 and 17. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to the emergency department complaining of back and abdominal pain associated with weight loss, constipation, and anemia. A computed tomography scan revealed a $12 \times 12 \times 10$ cm mass with displacement of the uterus, peritoneal carcinomatosis, ascites, and pelvic adenopathy. The CA125 level was 1971 U/mL. Her past medical history was significant for NF1 and two NF1-related tumors: a benign schwannoma excised from the breast 25 years previously and a T2bNOM0 PMNST of the right knee, treated with excision and radiation 7 years previously. A fine needle aspirate biopsy of the pelvic mass showed PAX8 (marker of Müllerian origin) positive HGSOc revealing a new primary gynecological malignancy, not a MPNST recurrence. The patient underwent radical tumor resection with total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and tumor resection, for optimal cytoreduction. Pathological review was performed per sectioning and extensively examining of the fimbriated end (SEE-FIM) protocols. HGSOc was noted in both ovaries and peritoneal metastasis with < 1% of the right fallopian tube (serosal side) involved. No serous tubal intraepithelial carcinomas were observed. Final pathological diagnosis was HGSOc arising from the ovary, stage IIB. After an uneventful post-surgery recovery, the patient refused adjuvant chemotherapy. Six months after surgery the patient experienced tumor progression and

began a course of dose dense carboplatin/paclitaxel. After 9 cycles of chemotherapy, CA125 levels normalized (< 5 U/mL), but a CT scan showed a residual 11×12 mm nodule in the pelvic mesentery. At the time of this manuscript, the patient is alive and receiving salvage chemotherapy for platinum-resistant progressive disease.

3. Results and discussion

Since malignant transformation of NF1-related cancers frequently involves early somatic mutation of the wild-type *NF1* allele followed by an additional genomic event (e.g. *TP53*, *CD2KNA* loss), we hypothesized that biallelic *NF1* inactivation was an early event in the development of HGSOc in this patient (Upadhyaya et al., 2004). To study tumor evolution, we performed WES on tumor samples obtained from each ovary and one peritoneal metastasis. Sequencing of germline DNA (average depth $130 \times$) revealed a missense mutation (c.7161C > G) in *NF1* and a deletion in *BRCA1* (c.1846_1848del), located in trans. The mutation in *NF1*, which leads to substitution of asparagine 2387 to lysine has not been previously reported and is characterized as a variant of unknown significance (VUS). However, based on the patient's clinical diagnosis and a previous report of a pathogenic *NF1* mutation involving

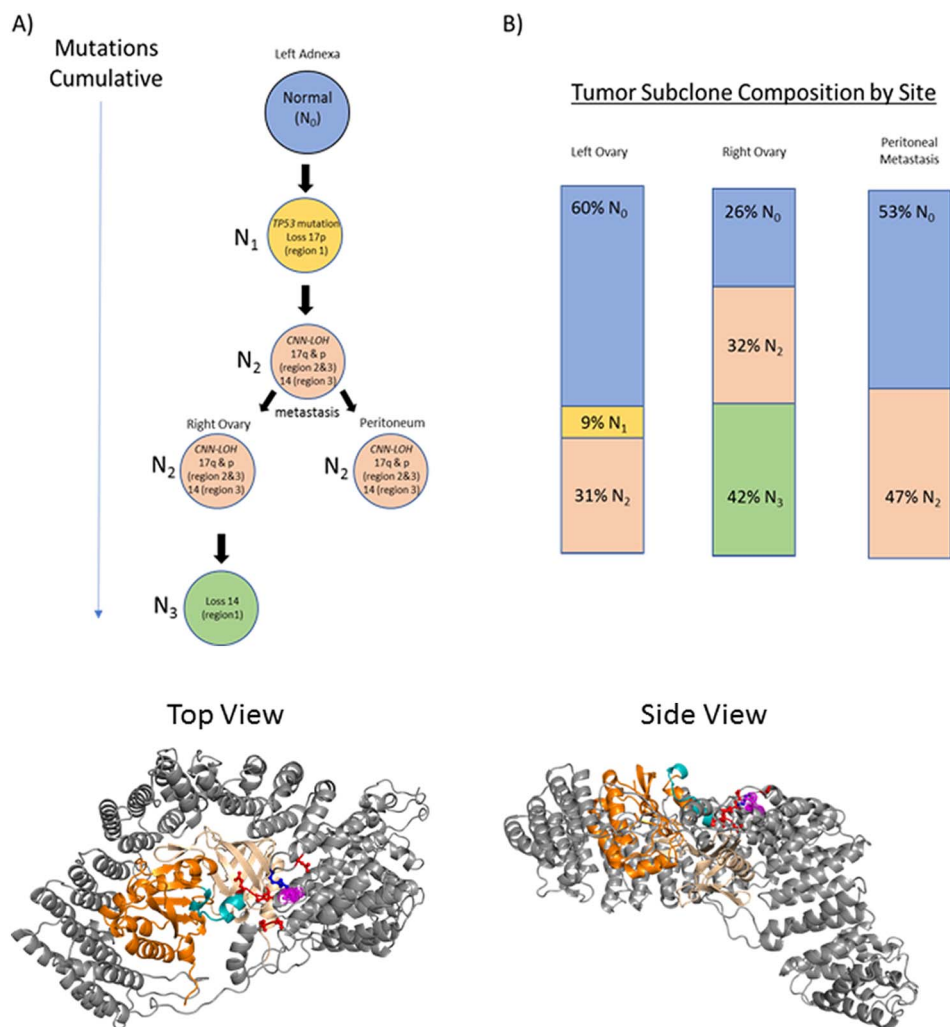


Fig. 2. Tumor clonal lineage and sequence of genomic events. (A) The mutational timeline of events is presented. Initial genomic alterations in an ancestral clone (N₁) located in the left adnexa included acquisition of a *TP53* mutation and losses of 17p&q. Subsequent copy-number neutral loss of heterozygosity (CNN-LOH) resulted in a dominant subclone (N₂) enriched at all three sites. An additional loss in region 1 of chromosome 14 produced an N₃ subclone only present in the right ovary sample. (B) Composition of each tumor sample as a percentage of normal tissue (N₀, blue) and tumor subclones N₁ (yellow), N₂ (pink), N₃ (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Structural model of neurofibromin CTD (grey) in the presence of the SEC14 (orange) and PH (beige) domains and the linker region (teal) between them. N2387 is shown in magenta. The acidic amino acid residues around N2387 are shown in red, whereas the basic amino acid residues around N2387 are shown in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in-frame deletion of N2387, as well as F2388 (ClinVar, M_001042492.2:c.7159_7164delAACTTT), we postulate that the *NF1* VUS reported herein represents a novel pathogenic mutation. Although, the *BRCA1* variant (ClinVar, NM_007294.3(BRCA1):c.1846_1848-delTCT (p.Ser616del)) has been previously described in women of African ancestry with early onset breast cancer (Biunno et al., 2014; Tazzite et al., 2012), there are conflicting reports of its pathogenicity. Analysis of the 1000 genomes project revealed this *BRCA1* variant (rs80358329) to be a rare variant observed in African sub-populations suggesting that it may be an ethnic specific polymorphism (Supplementary Fig. 1).

Sequencing of tumor samples (average depths 185 ×) revealed a marked degree of CNA and a frameshift mutation in *TP53* (p.T79 fs) in all tumor samples (Fig. 1, Supplementary Table 1). The CNA data, which includes regional consistencies in variant allele frequency (VAF) and comparative levels of sequencing depth, revealed a clonal tumor lineage which originated from an ancestral clone (N₁) in the left adnexa (Fig. 2). Initial genomic alterations associated with N₁, which accounts for ~40% of the tumor specimen located in the left ovary, included a copy loss event (thus LOH) in a portion of 17p&q (Fig. 1B, region 1) as well as a *TP53* mutation in the remaining allele. These events resulted in the N₁ tumor clone being homozygous for the *NF1* variant and the *TP53* mutation, and homozygous for the *BRCA1* reference allele. This is consistent with data from The Cancer Genome Atlas database, which demonstrates that somatic inactivation of *NF1* in HGSOc is frequently associated with CNA loss, whereas other cancer types exhibit mutational inactivation (Supplementary Fig. 2) (Cancer Genome Atlas

Research N, 2011). The bulk of the N₁-event associated tumor cells in the left ovary (78%) and possibly 100% of the tumor cells from other sites exhibited an additional copy number neutral LOH event (N₂) at 17q (regions 2 and 3) and chromosome 14 (region 3) (Fig. 1B). An additional subclone (N₃) was present in the right ovary and included a copy number loss in other regions of chromosome 14 (region 1) that occurred after metastasis but which clonally expanded to be the dominant clone at the time of surgery.

Based on calculation of the fraction of cells within the tumor that harbored key mutational events and potentially pathogenic germline variants (Supplementary Table 2), we concluded that the initial transformative events occurred in the left ovary and involved CN loss in a region of chromosome 17 that resulted in biallelic inactivation of *NF1* and loss of one *TP53* allele, as well as a *TP53* mutation (likely pathogenic) in the second allele. Since chromosome 17 CN loss and *TP53* mutation were present in similar cell fractions (i.e., overlapping confidence intervals) of the tumor at all three sites, we were unable to ascertain whether the two events occurred simultaneously or sequentially. Nevertheless, our findings provide evidence that biallelic inactivation of *NF1*, accompanied by inactivation of *TP53*, occurred early in the development of HGSOc.

To determine whether the N2387 K mutation located in the C-terminal domain (CTD) of neurofibromin could alter protein function, we performed sequence and structure analysis of the C-terminal region, which comprises of the SEC14, pleckstrin homology (PH) and C-terminal domains. Due to the large size of this region (~1250 amino acids) and availability of the X-ray crystal structure of only the SEC14 and PH

domains we used fold recognition techniques and structure based sequence analysis to identify in importin- β the closest template for building a putative coarse model of CTD using remote homology modeling techniques (Supplementary methods). This model suggested that the N2387K mutation could affect the interaction of the CTD with a highly acidic loop linking SEC14 and PH, thereby changing the relative configuration of these three domains to alter protein function (Fig. 3, Supplementary Methods and Supplementary Fig. 3). This finding provides further support for a pathogenic role of the N2387K mutation.

Forty-five percent of HGSOc show evidence of hyperactive RAS-signaling (Cancer Genome Atlas Research N, 2011). Since neurofibromin is a negative regulator of RAS-signaling, complete loss of *NF1*, as observed in this patient's tumor, could confer an advantage during the transformative process. Indeed, ovarian cancer cell lines that harbor *NF1* defects show increased RAS-mitogen activated protein kinase (MAPK) activation (Sangha et al., 2008). Our findings do not exclude the fact that *NF1* haploinsufficiency may contribute to HGSOc development. Notably, in vitro and in vivo studies demonstrated that *NF1* heterozygosity is associated with enhanced cellular proliferation and migration, as well as perturbed cellular differentiation. Further, *NF1*^{+/−} heterozygosity may modulate the microenvironment during tumorigenesis (Staser et al., 2010).

In summary, we highlight a case of a HGSOc patient with *NF1* which supports the concept that females with *NF1* should be monitored for ovarian cancer, in addition to breast cancer. The data presented suggest a causal link between *NF1* and HGSOc and provide a potential mechanism for the development of tumorigenesis, involving early biallelic inactivation of *NF1*. Notably, in this patient inactivation of *NF1* occurred in conjunction with *TP53* inactivation which is a necessary event for malignant transformation in HGSOc. Because *NF1* biallelic inactivation was ubiquitous in the tumor samples from this patient, our study suggests that inhibitors of RAS-signaling, including its downstream effectors, may represent a potential molecular target for these patients. Indeed, several ongoing clinical trials (e.g. The MILO Study, Clinicaltrials.gov, NCT01849874) are investigating the efficacy of MEK inhibitors in low grade serous ovarian carcinoma which frequently exhibit perturbed RAS-MAPK signaling. Thus, studies are warranted to determine if targeting RAS-signaling may provide a therapeutic benefit for patients with *NF1* that develop HGSOc.

Consent

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

EJN, RNG, and MKG designed the research study. EJN, DD, RNG, DT and MKG collected and processed tumor samples. DT and JK were involved in the clinical management of the patient. CL performed

pathological assessment of tumor samples. WDJ performed sequencing experiments. WDJ, IH and QZ performed analysis of sequencing data. MDS and AJP performed protein structural analysis. EJN, WDJ, and MKG wrote the manuscript. All authors edited the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gore.2018.01.005>.

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