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The KRAS-Variant Is Associated with Risk of Developing Double Primary Breast and Ovarian Cancer

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Abstract

Purpose: A germline microRNA binding site-disrupting variant, the KRAS-variant (rs61764370), is associated with an increased risk of developing several cancers. Because this variant is most strongly associated with ovarian cancer risk in patients from hereditary breast and ovarian families (HBOC), and with the risk of premenopausal triple negative breast cancer, we evaluated the association of the KRAS-variant with women with personal histories of both breast and ovarian cancer, referred to as double primary patients.

Experimental Design: Germline DNA from double primary patients was tested for the KRAS-variant (n = 232). Confirmation of pathologic diagnoses, age of diagnoses, interval between ovarian cancer diagnosis and sample collection, additional cancer diagnoses, and family history were obtained when available. All patients were tested for deleterious BRCA mutations.

Results: The KRAS-variant was significantly enriched in uninformative (BRCA negative) double primary patients, being found in 39% of patients accrued within two years of their ovarian cancer diagnosis. Furthermore, the KRAS-variant was found in 35% of uninformative double primary patients diagnosed with ovarian cancer post-menopausally, and was significantly associated with uninformative double primary patients with a positive family history. The KRAS-variant was also significantly enriched in uninformative patients who developed more than two primary cancers, being found in 48% of women with two breast primaries plus ovarian cancer or with triple primary cancers.

Conclusions: These findings further validate the importance of the KRAS-variant in breast and ovarian cancer risk, and support the association of this variant as a genetic marker for HBOC families previously considered uninformative.

Introduction

Hereditary breast and ovarian cancer (HBOC) syndrome is an inherited cancer-susceptibility syndrome marked by an increased risk of developing both ovarian cancer and breast cancer [1]. Families generally considered as having HBOC syndrome are those with multiple family members that have one of these cancers, especially at young ages, or an individual with a cancer in both organs, a “double primary” patient. While this is a relatively rare presentation, a substantial number of women develop both breast and ovarian primaries over their lifetime. While BRCA1 and BRCA2 are strongly associated with HBOC syndrome [2], a large number of HBOC families and women with double primary cancer do not havedetectable genetic mutations (herein referred to as “uninformative” patients).

The chances of identifying a mutation causative for HBOC increase when testing individuals diagnosed with double breast/ovarian primaries [3–5]. However, a recent report suggests that the rates of BRCA mutations are not higher in a patient with a double primary without a family history than that for isolated first
Prevalence of the KRAS-variant in Double Primary Patients by Ethnicity

Overall, the KRAS-variant was found in 21.0% of the entire cohort of double primary breast and ovarian cancer patients with full clinical information (n = 42/200). This is significantly higher than the population prevalence of ~15% observed in non-cancerous Caucasian control populations (p = 0.01 binomial test)[9–13]. Because the baseline prevalence of the KRAS-variant varies across ethnic populations [11], and is highest in Caucasian non-Hispanic populations, we examined the prevalence of the

<table>
<thead>
<tr>
<th>Table 1. The KRAS-variant is significantly associated with uninformative breast and ovarian cancer patients.</th>
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<tr>
<td><strong>BRCA1 (n = 75)</strong></td>
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<tr>
<td>Prevalence</td>
</tr>
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</table>

doi:10.1371/journal.pone.0037891.t001
The KRAS-variant Predicts Double Primary Cancer

**Table 2. The KRAS-variant is significantly more likely to be found in women tested within two years of their ovarian cancer diagnosis.**

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=62)</th>
<th>&lt;2 years from ovarian cancer diagnosis (n=52)</th>
<th>&gt;2 years from ovarian cancer diagnosis (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>30.5%</td>
<td>38.5%</td>
<td>16.7%</td>
</tr>
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</table>

KRAS-variant in Caucasian non-Hispanic double primary patients only, and found the prevalence of the KRAS-variant was slightly higher in these women compared to the overall cohort (38/163 = 23.3%, p = 0.002, binomial). The difference in prevalence of the KRAS-variant between Caucasian non-Hispanic, and non-Caucasian or Hispanic women with double primary cancer was not significant, however (p = 0.6), indicating that the KRAS-variant is significantly associated with double primary cancer for women of all ethnicities. Therefore all double primary patients, regardless of ethnicity, were included in the additional analyses.

### The Association of the KRAS-variant with BRCA Status

We evaluated the prevalence of the KRAS-variant in double primary patients with full clinical information based on BRCA mutation status: pathogenic BRCA1 mutations (n=75), pathogenic BRCA2 mutations (n=33), or BRCA-negative (uninformative) (n=92). The KRAS-variant was not statistically significantly elevated in women with pathogenic BRCA1 mutations (n=12/75, 16.0%), or in women with pathogenic BRCA2 mutations (n=6/33, 18.2%) compared to population prevalence. In contrast however, the prevalence of the KRAS-variant was significantly enriched in uninformative double primary cancer patients compared to population prevalence (25/92, 27.2%, p<0.001, binomial) (Table 1).

### Impact of Interval Between Ovarian Cancer Diagnosis and Patient Recruitment on KRAS-variant Prevalence in Uninformative Patients

Because the KRAS-variant predicts poor ovarian cancer specific survival in uninformative patients [14], we investigated the association of the prevalence of the KRAS-variant and time between ovarian cancer diagnosis and study recruitment for uninformative patients with available information (n=82). First, we found that the interval between ovarian cancer diagnosis and sample collection was significantly different across the recruitment centers, likely due to center referral patterns (p<0.001). The overall prevalence of the KRAS-variant was 30.5% (n=25/82) in uninformative patients with available information on interval between diagnosis and recruitment. The prevalence of the KRAS-variant was 38.5% (n=20/52) in patients recruited within two years of their ovarian cancer diagnosis, which was significantly higher than the prevalence in patients recruited more than 2 years after their ovarian cancer diagnosis (16.7%, n=5/30, p<0.048 by Exact test) (Table 2).

### Timing of Ovarian Cancer Development in KRAS-variant Uninformative Patients

The majority of uninformative women in these studies developed breast cancer before their ovarian cancer (74.7% of all uninformative patients [n=65/87]). This was slightly less common in KRAS-variant-positive uninformative patients (64%, n=16/25) compared to KRAS-variant-negative uninformative patients (79.0%, n=49/62), but this difference was not significant. Because prior reports have found that the KRAS-variant is rarely associated with premenopausal ovarian cancer (less than 52 years of age) [9,14], we next evaluated the association of the KRAS-variant with age of ovarian cancer development in uninformative double primary patients. We found that 88.0% of KRAS-variant-positive uninformative patients developed ovarian cancer postmenopausally (n=22/25), compared to only 66.1% of KRAS-variant-negative uninformative patients (n=41/62), however this difference did not reach statistical significance (p = 0.062). We additionally found a significant association of the KRAS-variant with age of ovarian cancer diagnosis, with 34.9% of women diagnosed with ovarian cancer postmenopausally having the KRAS-variant (n=22/63), compared to only 12.5% of women diagnosed with ovarian cancer premenopausally (n=3/24). This association with older age of ovarian cancer onset in KRAS-variant-positive uninformative patients was significant by logistic regression analysis (p<0.007) (Table 3).

### Association of the KRAS-variant with Family History in Uninformative Patients

As the association of double primary cancers and known genetic mutations has been found to be enriched in the presence of a positive family history of related cancers, we evaluated the association of the prevalence of the KRAS-variant with family history in uninformative patients. We added an additional cohort of 32 uninformative double primary patients with a known family history to the 44 uninformative patients with known family history from our fully annotated cohort. In these 76 women with double primary cancers, 24 had a positive family history and 52 had a negative family history for breast and/or ovarian cancer in first and/or second-degree relatives. The KRAS-variant was found in 29.2% (7/24) of women with a positive family history, which is a

**Table 3. The KRAS-variant is significantly associated with developing ovarian cancer post-menopausally compared to pre-menopausally.**

<table>
<thead>
<tr>
<th></th>
<th>Women with post-menopausal ovarian cancer (n=63)</th>
<th>Women with pre-menopausal ovarian cancer (n=24)</th>
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</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>34.9%</td>
<td>12.5%</td>
</tr>
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[Image 58x24 to 76x41]

doi:10.1371/journal.pone.0037891.t002

Table 1

Table 2

Table 3
prevalence significantly higher than expected in the general population \(p<0.02\). In contrast, the \textit{KRAS}-variant was not significantly elevated in uninformative double primary patients with a negative family history compared to the general population prevalence, being found in 15.3\% \((8/52)\) of this population. The difference between the prevalence of the \textit{KRAS}-variant in women with a positive versus negative family history was not significant \((p = 0.13)\).

**Association of the \textit{KRAS}-variant with Multiple Cancers in All Patients**

Because the \textit{KRAS}-variant has been found to be associated with an increased risk for other cancers besides breast and ovarian cancer \[11,15\] we tested the hypothesis that the \textit{KRAS}-variant would predict for an increased risk of developing additional cancers in this double primary cohort, regardless of \textit{BRCA} mutation status. For 183 of the patients in our study where this information was available, 79.2\% \((n = 145)\) had reported just the two cancers (breast and ovarian), 12.0\% \((n = 22)\) had two separate primary breast cancers and also ovarian cancer, and 8.7\% \((n = 16)\) had cancer in an additional organ outside of the breast and ovary (triple primary).

The \textit{KRAS}-variant was found in 20.0\% \((n = 29/145)\) of double primary patients overall; 19.3\% \((11/57)\) of \textit{BRCA1} patients, 13.6\% \((3/22)\) of \textit{BRCA2} patients and 22.7\% \((15/66)\) of uninformative patients. The \textit{KRAS}-variant was found in 22.7\% \((n = 5/22)\) of patients with two separate primary breast cancers and ovarian cancer; 0\% \((0/12)\) of \textit{BRCA1} patients, 33.3\% \((1/3)\) of \textit{BRCA2} patients and 57.1\% \((4/7)\) of uninformative patients. Finally, the \textit{KRAS}-variant was found in 43.8\% \((n = 7/16)\) of women with triple primaries; 0\% \((0/1)\) of \textit{BRCA1} patients, 100\% \((1/1)\) of \textit{BRCA2} patients, and 42.9\% \((6/14)\) of uninformative patients. The \textit{KRAS}-variant predicts a significant increased risk of developing a third independent cancer in all double primary patients \((p<0.01)\), which was largely due to increased risk for uninformative patients \((p<0.005)\) and also possibly \textit{BRCA2} patients \((p<0.05)\). The \textit{KRAS}-variant also predicts a significantly increased risk of developing more than two primary cancers in uninformative double primary patients, being found in 47.6\% \((10/21)\) of uninformative patients with more then two primary cancers compared to 22.7\% \((15/66)\) of uninformative patients with just two primary cancers \((p = 0.05)\) (Table 4).

**Discussion**

Here we show that the \textit{KRAS}-variant, a functional germline miRNA-binding disrupting mutation that has previously been shown to be associated with ovarian cancer, especially in HBOC families \[9\], as well as with premenopausal triple negative breast cancer \[10\], is also significantly enriched in women who develop both breast and ovarian cancer with uninformative \textit{BRCA} sequencing results (Table 5). The \textit{KRAS}-variant was most enriched in women who were tested within two years of their ovarian cancer diagnosis, likely reflecting the increased risk of intermediate death of \textit{KRAS}-variant positive ovarian cancer patients with longer accrual times \[9\]. In addition, the \textit{KRAS}-variant was significantly associated with \textit{BRCA}-uninformative patients who developed ovarian cancer post-menopausally (as estimated by age >52 years), and with \textit{BRCA}-uninformative patients with a positive family history of breast or ovarian cancer. Finally, the \textit{KRAS}-variant was significantly associated with an increased risk of developing a third, independent cancer in addition to breast and ovarian cancer, being found in 43.8\% of patients with triple primary cancers, most of whom had uninformative \textit{BRCA} testing. It is possible that a small proportion of cases considered \textit{BRCA}-uninformative may harbor a large rearrangement mutation, known to account for about 10\% of deleterious \textit{BRCA} mutations \[16,17\] given the lack of screening in many cases. However, this would not have altered the significance of the primary observations in this report. These findings further confirm that the \textit{KRAS}-variant is indeed a bona fide marker for uninformative HBOC families, and also highlights some similarities as well as some differences between \textit{KRAS}-variant patients and \textit{BRCA} mutant patients.

Because the great majority of \textit{KRAS}-variant double primary patients in this study developed breast cancer before their ovarian cancer, it appears that there could have been an opportunity for ovarian cancer prevention through chemoprevention (oral contraceptives) and/or prophylactic oophorectomy for these women. In addition, the association of the \textit{KRAS}-variant primarily with postmenopausal ovarian cancer suggests that oophorectomy might be reasonable delayed in these patients compared to recommendations for women with \textit{BRCA} mutations, where oophorectomy is recommended at 35 or upon completion of childbearing. Currently, women with premenopausal breast cancer who are uninformative for \textit{BRCA1} mutations without a family history of ovarian cancer are told that they have no increased risk of ovarian cancer, based on a study of hereditary breast cancer families \[7\]. Our findings here indicate that women with the \textit{KRAS}-variant are also at an increased risk of subsequently developing ovarian cancer, and should be managed accordingly.

### Table 4. The \textit{KRAS}-variant is significantly associated with the risk of developing additional cancers beyond breast and ovarian cancer.

<table>
<thead>
<tr>
<th></th>
<th>Breast and ovarian cancer ((n = 143))</th>
<th>Two breasts and ovarian ((n = 22))</th>
<th>Triple primary cancer ((n = 14))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence overall</td>
<td>20.0%</td>
<td>22.7%</td>
<td>43.8%</td>
</tr>
<tr>
<td>Prevalence in uninformative</td>
<td>22.7% ((15/66))</td>
<td>57.1% ((4/7))</td>
<td>42.9% ((6/14))</td>
</tr>
</tbody>
</table>

**Table 5. Prevalence of the \textit{KRAS}-variant in uninformative patients.**

<table>
<thead>
<tr>
<th></th>
<th>YES ((n = 145))</th>
<th>NO ((n = 145))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accrued within 2 years of ovarian cancer diagnosis</td>
<td>38.5%</td>
<td>16.7%</td>
<td>0.048</td>
</tr>
<tr>
<td>Developed ovarian cancer post-menopausally</td>
<td>34.9%</td>
<td>12.5%</td>
<td>0.007</td>
</tr>
<tr>
<td>Developed more than two primary cancers</td>
<td>47.6%</td>
<td>22.7%</td>
<td>0.05</td>
</tr>
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</table>

[doi:10.1371/journal.pone.0037891.t004][1]

[doi:10.1371/journal.pone.0037891.t005][2]
The finding that the prevalence of the KRAS-variant is significantly higher in women tested within two years of ovarian cancer diagnosis likely reflects the fact that these patients have worse ovarian cancer specific survival and a higher risk for interim death over time [14]. In addition, the significant association of the KRAS-variant with early onset triple negative breast cancer [10], the most deadly form of breast cancer, would also have likely diluted the prevalence of the KRAS-variant in these cohorts, as these women would be more likely to die of their breast cancer before development of ovarian cancer. Regardless, the prevalence of the KRAS-variant remained significantly enriched in these patients even when studying the group as a whole. Importantly though these findings highlight the necessity of carefully considering study design when analyzing markers that predict aggressive tumor biology, such as the KRAS-variant. Erroneous conclusions will otherwise be reached when using prevalence as a measure of the association with cancer risk if the populations studied have long ascertainment times. Such disparities in these and other areas of study cohort and design likely explain the failure to find the association between the KRAS-variant and sporadic ovarian cancer risk in a prior publication [13]. However, it is also important to highlight that the association found in this study is again strongest if the populations studied have worse ovarian cancer specific survival and a higher risk for interim cancer diagnosis likely reflects the fact that these patients have significantly higher in women tested within two years of ovarian cancer.

The finding that the KRAS-variant is associated with uninformative women with double primary cancer is important, as it further confirms that 1) the KRAS-variant is associated with uninformative HBOC families, 2) appropriate intervention for patients with the KRAS-variant who develop breast cancer may allow prevention of future ovarian cancer and 3) women with cancer that have the KRAS-variant may benefit from screening to detect additional cancer development at its earliest stages. Overall, this work continues to support the importance of the KRAS-variant broadly in cancer biology, and specifically in women’s health.

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Author Contributions
Conceived and designed the experiments: RP JBW JW LS. Performed the experiments: TM JD HH NM MH SS. Analyzed the data: DP DZ. Contributed reagents/materials/analysis tools: RP JW MK XW LS MM. Wrote the paper: RP DP JW TM JD HH NM JBW MK MM XW MH DZ SS LS.

References