Gene panel testing for breast cancer should not be used to confirm associations with syndromic genes in particular for \( NF1 \) pathogenic variants

Running Head: Panel testing for syndromic gene cancer associations

D. Gareth Evans MD,\(^1\),\(^2\),\(^3\) Sacha J Howell PhD,\(^3\),\(^4\) Ian M. Frayling MD PhD,\(^5\),\(^6\) Juha Peltonen MD, PhD\(^7\)

\(^1\)Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, M13 9WL, UK.

\(^2\)Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.

\(^3\)The Christie NHS Foundation Trust, Wilmslow Road, Manchester, M20 4BX, UK.

\(^4\)Division of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.

\(^5\)All-Wales Medical Genetics Service, Cardiff & Vale University Health Board, University Hospital of Wales, Cardiff, CF14 4XW, UK.

\(^6\)Institute of Medical Genetics, Division of Cancer & Genetics, School of Medicine, Cardiff University, Cardiff, CF14 4XN, UK.

\(^7\)Department of Cell Biology and Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland

Correspondence: Prof DG Evans, Department of Genetic Medicine, Manchester Academic Health Sciences Centre (MAHSC), St Mary’s Hospital, University of Manchester, Manchester M13 9WL Email: gareth.evans@mft.nhs.uk
Tel: +44 (0)161 276 6506; Fax: +44 (0)161 276 6145

Conflict of interest: None to declare

Word count 1105
In recent years there has been an explosion in the use of multi-gene panels to test for cancer predisposition often utilising large panels across many tumour types. More recently the results of these tests have been used as a form of case control study to assess genes for cancer associations, especially with breast cancer. Case control analysis is arguably the most informative method to identify gene-cancer associations as it also provides confirmation of the level of any increased risk. Ideally the ‘cases’ should be derived from a truly unselected series of individuals with the relevant cancer. Similarly the controls should come from the same population, and can be either true ‘population’ controls from representative, unselected and ideally age matched individuals, or ‘super’ controls (older/age matched individuals known not to have the malignancy being investigated). Particularly for rare diseases (present in less than 1 in 2000 individuals), very large case control series are required to confirm moderate risk elevations of only 2-3 fold. Whilst traditional methods such as positional cloning from family linkage were used for identifying the BRCA1 and BRCA2 genes in 1994 and 1995, most other breast cancer predisposition genes were identified as causal from candidate gene approaches using case control series often enhanced by using familial samples. However, even these have identified potentially spurious associations, because analyses of breast/ovarian cancer families has identified a real association with ‘ovarian’ cancer, but a potentially false association with breast cancer\(^1\). In particular, initial breast cancer associations with RAD51C, RAD51D and BRIP1 were later called into question with breast cancer specific analysis\(^1\). Hence, these three genes are not on the UK’s National Health Service breast cancer panel,\(^3\) but they do appear on most commercial ‘breast cancer’ specific panels. Other DNA repair gene associations, such as for ATM, CHEK2 and PALB2, have been well validated in multiple cohort studies and added to breast cancer specific gene panels.\(^3\) In contrast to the aforementioned genes which have no recognisable syndromic phenotype for an individual, the phenotypes from germline pathogenic variants in NF1, PTEN and STK11 (table 1) are usually easily recognisable in single individuals and there are well validated diagnostic criteria which allow a clinical diagnosis without the need for molecular confirmation.\(^4\)\(^,\)\(^5\)

Four recent articles based on multi-gene panel testing, published in high impact oncology journals, have nonetheless concluded that there are no associations between pathogenic variants in these three syndromic genes (NF1, PTEN and STK11) and breast cancer risk.\(^6\)\(^-\)\(^9\) Whilst these analyses have identified potential new gene associations, the negative results concerning syndromic associations should be tempered. In the first of their three articles Ambry’s 21 panel gene test\(^6\) was evaluated in 41,611 consecutively tested white women with breast cancer. In the second\(^7\) 9,639 patients with breast cancer were assessed, whilst the third assessed the risk of triple negative breast cancer in 8,753 women. Whilst two studies used control data from the ExAc database\(^6\)\(^,\)\(^8\) the second used a combination of controls tested for non-cancer indications.\(^7\) The larger initial study\(^6\) identified NF1 gene variants in 0.1% compared to 0.11% in ExAc controls. No control frequency was provided for the second study although a frequency of around 0.15% was said to be non-significant\(^8\). The third study also found a frequency of 0.15% in triple negative breast cancer which was also non-significant.\(^8\) The first two studies effectively excluded BRCA1 and BRCA2 as they confirmed that there had been a high degree of pre-testing for these genes. The first also excluded what they ‘termed’ ‘syndromic’ genes including PTEN, CDH1 and TP53. However, it is unclear why neurofibromatosis 1, caused by pathogenic variants in the NF1 gene, was not also excluded as being
syndromic, as it is far more recognisable from patient characteristics than even PTEN hamartoma syndrome.⁴

There are two main flaws in the conclusions that NF1 is not associated with breast cancer. The first is that panel testing for breast cancer is selective based on family history, age and patient/clinician choice. A clinician who already has an ‘explanation’ for a breast cancer in a patient with NF1 is unlikely to send off for a gene panel. Indeed the syndromic learning problems associated with NF1 may also preclude gainful employment and thus reimbursement for panel testing. The link to breast cancer based on cohort studies is now irrefutable with six studies reporting Odds Ratios of 4-11 fold for NF1 women aged <50 years of age⁴,¹¹. Furthermore, driver NF1 pathogenic variants have been identified in the Cancer Genome Atlas and from NF1 patients are associated with higher tumour grade and Human Epidermal Growth Factor Receptor 2 (HER2) overexpression,¹⁰,¹² further evidence against a chance association. The link with breast cancer and NF1 has been established since at least 2007¹¹. All of the reported panel tests were performed since March 2012⁶-⁸, after four of the cohort studies had reported showing a probable causal association with breast cancer and NF1¹¹. The authors of the Ambry reports clearly admit that there was already preselection for BRCA testing⁶-⁸. If a clinician wished to test for NF1 they would use a substantially more sensitive RNA based approach than a panel¹⁴. A similar concern should also be expressed for the apparent lack of association with PTEN in the second and third Ambry studies⁷,⁸ as these were not powered to assess the lower frequency of PTEN pathogenic variants and suffer from the same issue of lack of requirement for a panel test when an explanation for breast cancer was already present. The same criticism can be put forward for the absence of STK11 variants being identified in 2000 familial breast cancer samples tested in the LIFEPOOL study.⁹ Peutz-Jeghers disease caused by STK11 variants is easily identified by peri-oral pigmentation and usually presents symptomatically in early life with multiple intestinal polyps (Table 1).

The second potential flaw is the use of ExAc controls. The very high frequency of apparent pathogenic variants of 1/900 is simply not consistent with the estimates of birth incidence from highly ascertained populations of between 1 in 2,000-2,600¹⁵,¹⁶. Due to early deaths in NF1 patients prevalence in an adult population is nearer 1 in 3,000-4,500.¹⁵,¹⁶ It is therefore unclear whether this high estimate in controls is due to over assessment of pathogenic variants, selection for children to have exomes with NF1 features or due to some variants being silent clinically.

Whilst panel tests appear to be a useful agnostic test for cancer associations with non-syndromic genes, they are not when considering easily recognisable syndromes, as these create biases in selection against any association and may also contaminate controls. The only way to robustly assess for links with syndromes in a case control study is for ALL patients with breast cancer to be tested on a population basis with appropriate controls tested of a similar age, also without selection.

**Acknowledgements**

DGE and SJH are supported by the all Manchester NIHR Biomedical Research Centre (IS-BRC-1215-20007). JP is supported by Cancer Society of Finland.
References

3. Taylor A, Brady AF, Frayling IM on behalf of the UK Cancer Genetics Group (UK-CGG), et al. Consensus for genes to be included on cancer panel tests offered by UK genetics services: guidelines of the UK Cancer Genetics Group Journal of Medical Genetics 2018;55:372-377
Table 1: Syndromic genes identifiable from clinical features in a single woman that increase breast cancer risk from cohort studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Birth incidence</th>
<th>Clinical features</th>
<th>Breast cancer risk to 50 yrs/lifetime</th>
<th>Other malignancy risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>Neurofibromatosis 1</td>
<td>1 in 2,000-2,500</td>
<td>Café au lait, Cutaneous neurofibromas, Iris Lisch nodules</td>
<td>10%/20%</td>
<td>Malignant peripheral nerve sheath tumor, glioma</td>
</tr>
<tr>
<td>PTEN</td>
<td>PTEN hamartoma syndrome (Cowden)³</td>
<td>1 in 100,000-200,000</td>
<td>Macrocephaly, mucocutaneous lesions (eg Trichilemmomas)</td>
<td>50%/85%</td>
<td>Thyroid, endometrial</td>
</tr>
<tr>
<td>STK11</td>
<td>Peutz-Jeghers⁴</td>
<td>1:25,000 to 1:280,000</td>
<td>Peri-oral pigmentation, hamartomatous bowel polyps</td>
<td>Nk/37-55%</td>
<td>Colorectal, stomach, small bowel, ovary, cervix, pancreas, testes</td>
</tr>
</tbody>
</table>