Review

Testing for CHEK2 in the cancer genetics clinic: ready for prime time?

The 1100delC mutation of the CHEK2 gene was found to be a cause of breast cancer in 2002. The lifetime risk of breast cancer among women with a mutation and with a family history of breast cancer is approximately 25%. These women are good candidates for screening with MRI and for chemoprevention with tamoxifen. It is reasonable to test for this single mutation when women undergo testing for BRCA1 and BRCA2.

Genetic testing is widely available in North America and Europe for two breast cancer susceptibility genes, BRCA1 and BRCA2 (1). Because these two genes do not explain all breast cancer families, it is expected that additional genes will be discovered. However, since BRCA1 was identified in 1994 and BRCA2 was identified in 1995 (2, 3) progress in identifying these genes has been slow. A few other highly penetrant breast cancer genes have been found, including p53, BRIP1 and PALB2 (4–6), but families with mutations in these are exceedingly rare. Other cancer susceptibility genes have been discovered through genome-wide association studies, but the risk of breast cancer for women who carry a susceptibility allele in one of these genes is too low to generate family clusters (7). Arguably, the most relevant of the post-BRCA genes, from a clinical point of view, is CHEK2, which was first linked to breast cancer susceptibility in 2002 (8). However, in the eight years since this seminal discovery, clinical testing for CHEK2 mutations has gained little support. It is instructive to consider why this might be the case.

Conditions for genetic testing

For a cancer genetic test to reach the clinic, several conditions should be met. Mutations must be sufficiently common among women who are tested that a positive result is not a rare occurrence. The test must be relatively inexpensive and it should be clear which variants are deleterious and which are benign. It is important to have an identifiable target population of women to test, i.e. to know roughly, what is the probability that a given women will have a positive test? It is important to have knowledge of the range of cancers associated with a mutation and to know the lifetime risk of each type of cancer. Women go to a cancer genetics clinic because of a family history of cancer, and if the gene penetrance is not sufficiently high to cause familial clustering, then mutation carriers will not identify themselves as candidates for testing. Women are referred to counsellors (or self-refer) to seek an explanation for their personal or family history of cancer, and not to request a specific molecular test. If the lifetime cancer risk is not substantially increased beyond that of a woman with a normal result, few women will be motivated to undergo genetic testing. Finally, it is important to know what factors may influence the risk of cancer, in particular those which are modifiable. For these conditions to be met, genetic epidemiology studies must be conducted. Because most gene mutations are rare in the general population, genetic epidemiology studies...
are almost always conducted on individuals with cancer, or on cancer families. If a mutation is present in only a very small fraction of women with cancer, say 1%, then one would need to test 10,000 unselected cases to identify 100 carriers of the mutation.

**CHEK2 and breast cancer**

To date, no breast cancer susceptibility gene has been discovered since *BRCA1* or *BRCA2* that satisfies all of these conditions, but the closest candidate is *CHEK2*. A recurrent mutation in the *CHEK2* gene (1100delC) was reported to be a cause of breast cancer in 2002 by Meijers-Heijboer et al. (8). Numerous association studies have since confirmed this association (9–21). The prevalence of the 1100delC mutation varies widely between countries, but the odds ratios reported for breast cancer for women with this single mutation are consistently between 1.5 and 3.0. A meta-analysis of all association studies estimated the risk of breast cancer among carriers to be increased by a factor of 2.7 (14). The mutation is responsible for about 3% of breast cancer cases in the Netherlands, and is less frequent in other Northern European countries (Table 1). The 1100delC allele does not seem to be present in Southern Europe or in most non-white populations (20, 22–24) (there may be other deleterious alleles in these and other ethnic groups, but these have not yet been discovered). The 1100delC mutation is present in 0.5% of Polish breast cancer patients (21).

There are two other protein-truncating founder mutations in *CHEK2* which are present in the Slavic populations of Eastern Europe (IVS2+1G>A; del5395) (21, 25–28). The IVS2+1G>A has been reported in Poland (21), Belarus (26) and Russia (27). The del5395 mutation has been reported in Poland (21), Belarus (26) and the Czech Republic (28). The contribution of these alleles to the overall burden of breast cancer in Slavic populations has not been well studied, with the exception of Poland. One of the three *CHEK2* truncating mutations (1100delC, IVS2+1G>A, del5395) accounts for approximately 2.3% of all early-onset breast cancer cases in Poland (21).

**CHEK2 and other cancers**

In 2003, Dong and colleagues first reported an association between *CHEK2* mutations and prostate cancer (29) and this has since been confirmed by others (30, 31). The odds ratio for prostate cancer, given the 1100delC mutation is estimated to be about 3.0 (30, 31). Similar estimates have been reported for the other two truncating mutations (31). The full range of cancers associated with the 1100delC *CHEK2* mutation has not yet been fully delineated, but there is evidence that it increases the risk for cancers of the thyroid and kidney (32). It has been suggested that the *CHEK2* 1100delC mutation was associated with colorectal cancer in Holland (33), but this association has not been replicated (34–37).

**I157T missense mutation**

There is a missense variant in *CHEK2* which, in Northern and Eastern Europe, is much more common than the 1100delC allele (I157T) (32, 38–40). The full extent of the geographic distribution of the I157T allele has yet to be determined. This variant was originally reported to be a non-deleterious polymorphism; however, more recent studies on large patient samples indicate that it predisposes to various types of cancer (32). The I157T variant has been associated with breast cancer in Poland, Finland, Germany and Belarus (21, 25, 38). The odds ratio for breast cancer associated with this mutation is approximately 1.5 (21, 38). In a series of papers from Poland, Cybulski et al. estimated the risks of cancer of the breast, colon, prostate, associated with the I157T missense mutation, and compared these risks with the risks associated with the three truncating alleles (Table 2) (21, 31, 32, 39). The odds ratio associated with the I157T allele was about 1.5 for each of the cancer types.

<table>
<thead>
<tr>
<th>Country/ethnic group</th>
<th>Number of cases</th>
<th>% Positive</th>
<th>Number of controls</th>
<th>% Positive</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Netherlands</td>
<td>962</td>
<td>2.9</td>
<td>184</td>
<td>1.6</td>
<td>1.8</td>
<td>0.5–6.0</td>
<td>10</td>
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<tr>
<td>Finland</td>
<td>1035</td>
<td>2.0</td>
<td>1885</td>
<td>1.4</td>
<td>1.5</td>
<td>0.8–2.6</td>
<td>15</td>
</tr>
<tr>
<td>Denmark</td>
<td>1101</td>
<td>1.1</td>
<td>4655</td>
<td>0.5</td>
<td>2.6</td>
<td>1.3–5.4</td>
<td>9</td>
</tr>
<tr>
<td>Germany</td>
<td>613</td>
<td>0.8</td>
<td>417</td>
<td>0.5</td>
<td>1.7</td>
<td>0.3–8.8</td>
<td>12</td>
</tr>
<tr>
<td>Poland</td>
<td>1978</td>
<td>0.5</td>
<td>5496</td>
<td>0.2</td>
<td>2.3</td>
<td>1.0–5.4</td>
<td>21</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>688</td>
<td>0.4</td>
<td>730</td>
<td>0.3</td>
<td>1.6</td>
<td>0.3–8.8</td>
<td>11</td>
</tr>
<tr>
<td>French-Canadians</td>
<td>564</td>
<td>1.1</td>
<td>6424</td>
<td>0.3</td>
<td>3.6</td>
<td>1.4–9.1</td>
<td>20</td>
</tr>
<tr>
<td>Jews</td>
<td>320</td>
<td>1.2</td>
<td>180</td>
<td>–</td>
<td>–</td>
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</table>
In contrast, the 1100delC mutation increases the risk of breast and prostate cancer by approximately threefold, but had no effect on colon cancer risk (37, 39). The CHEK2 I157T missense variant, but not the 1100delC mutation, has also been associated with colorectal cancer in Finland and the Czech Republic (38, 40).

In a study of Polish and Russian patients, positive associations were seen with the CHEK2 I157T missense variant and ovarian cystadenomas (OR = 1.7; p = 0.005), with borderline ovarian cancers (OR = 2.6; p = 0.002) and with low-grade invasive cancers (OR = 2.1; p = 0.04), but not with high-grade invasive cancers (41).

### Lung cancer

In 2008, Brennan et al. (42) reported the surprising result that the CHEK2 I157T mutation appears to protect against lung cancer in patients from Eastern Europe (OR = 0.44; 95% CI 0.31–0.63; p = 0.00007). This large protective effect was confirmed shortly thereafter in a second sample of 895 lung cancer patients from Poland (OR = 0.3; 95% CI 0.2–0.5; p = 0.00001) (43). The missense I157T mutation was equally protective against lung cancer as were the three truncating mutations. The presence of a CHEK2 mutation also appears to protect against laryngeal cancer (OR = 0.6; 95% CI 0.3–0.99; p = 0.05) (43).

### Risk modifiers in CHEK2 carriers

For carriers of a mutation in BRCA1, BRCA2 or CHEK2, the risk of breast cancer for a woman with a positive family history of breast cancer is greater than that for carrier of the same mutation, but who has no family history of breast cancer (44–48). The effect is particularly strong for carriers of CHEK2 mutations; for this reason, CHEK2 has been described as a modifier of risk of other susceptibility genes (46–48). In practical terms, a genetic counselor should give a relatively high estimate of the lifetime risk of cancer to a carrier of a CHEK2 mutation if her family history is strong. CHEK2 mutations are found among familial breast cancer cases, but surprisingly often, the mutation does not segregate with the cancer phenotype. Again, this suggests that CHEK2 modifies the presence of another (unidentified) cancer susceptibility gene (i.e. the one that caused the family cluster). Genetic counseling for CHEK2 is problematic because the risk of cancer depends not on the mutation status alone, but also on the patient’s family history. Gronwald et al. estimated the cumulative incidence of breast cancer in the female first-degree relatives of 533 probands with a CHEK2 mutation (45). If the proband had breast cancer, the cumulative incidence was 10.4% to age 75. If the proband had another type of cancer, the cumulative incidence was 3.6% (HR = 3.6; p < 0.001 for difference).

CHEK2 mutations are generally not found in cancer families which segregate a mutation in BRCA1 (20) and therefore CHEK2 does not appear to be a risk modifier of BRCA1. In fact, Cybulski et al. found the opposite – the missense CHEK2 variant I157T seems to protect against the development of breast cancer in carriers of a BRCA1 mutation (49). The odds ratio for the BRCA1 mutation alone was 13.1 (95% CI 8.2–2.1). The odds ratio for carriers of both mutations (compared to neither) was 6.6 (95% CI 1.5–2.9). In contrast to the situation with BRCA1, Serrano et al. (50) reported a synergistic effect between CHEK2 mutations and a missense variant in BRCA2. In the absence of a CHEK2 mutation, the BRCA2 variant was associated with a significant reduction in the risk of breast cancer (OR = 0.62; p = 0.0007) but coupled with a CHEK2 mutation, the odds ratio was 5.7 (95% CI 1.7–19). A similar synergistic effect has been reported for CHEK2 mutations and p27 variants and prostate cancer susceptibility (51). In this study by Cybulski et al., the excess risk of prostate cancer associated with a CHEK2 mutation was restricted to the subgroup of men who were homozygous for the VV variant allele in codon 109 of the p27 gene. Among men with the VV p27 genotype, the odds ratios associated with truncating and missense CHEK2 mutations were 3.1 (p < 0.0001) and 1.9 (p < 0.0001), respectively. Among men with other p27 genotypes (GG and VG), the odds ratios were 1.5 and 1.2 for truncating and missense CHEK2 mutations, respectively, and were not statistically significant. It is likely that other genes will be discovered for which CHEK2 is a modifier.

<table>
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<th>Reference</th>
<th>Table 2. Odds ratios associated with truncating and missense CHEK2 mutations in Poland*</th>
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<tr>
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<td>Truncating mutations OR (95% CI)</td>
</tr>
<tr>
<td>Breast</td>
<td>3.3 (2.4–4.3)</td>
</tr>
<tr>
<td>Prostate</td>
<td>2.5 (1.6–3.7)</td>
</tr>
<tr>
<td>Colon</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>Lung</td>
<td>0.4 (0.1–1.3)</td>
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*The truncating mutations included 1100delC; del5395 and IVS2+1G>A. The missense mutation was I157T.
Narod

**CHEK2 and breast cancer: clinical implications**

The clinical management of women with a CHEK2 mutation depends on several factors, including the lifetime risk of breast cancer, the age distribution of the cancers, the characteristic pathology, the natural history, and the response to treatment. The lifetime breast cancer risk for women with an 1100delC mutation and a first-degree relative with breast cancer is approximately 25%. Few patients will consider preventive mastectomy at this level of risk.CHEK2 1100delC carriers are not at increased risk for ovarian cancer and prophylactic oophorectomy has not been shown to reduce the risk of breast cancer in CHEK2 carriers. However, at this level of breast cancer risk, annual MRI screening is appropriate (52).

Also, women with and without cancer should be considered to be candidates for tamoxifen. To date, no specific studies have been done to measure the effectiveness of tamoxifen in CHEK2 carriers, and for logistical reasons, it is unlikely that this information is forthcoming; therefore, the decision to give tamoxifen must be based on studies of other populations (53). The majority of breast cancers which arise in CHEK2 carriers are estrogen-receptor (ER)-positive (54–56). In a Dutch study, 80% of the CHEK2 cases were positive for ER, and in Poland 72% of the patients were ER-positive (54, 55). The cancers in CHEK2 carriers do not occur at a particularly young age and it is reasonable to wait until age 40 to initiate a course of tamoxifen. CHEK2 carriers with breast cancer are also at high risk of a second primary breast cancer and it is reasonable to consider tamoxifen prophylaxis to prevent these as well. Other than ER status, CHEK2-associated tumors do not appear to be appreciably different from tumors in mutation-negative women (56–58). Little is known about the natural history of CHEK2-associated breast cancers. Two studies reported that 1100delIC allele is a predictor of adverse prognosis and of poorer survival (57, 59). To date, no studies have been done on the influence of a CHEK2 mutation on the response to chemotherapy in breast cancer patients, and currently, treatment should be based on conventional criteria.

**Testing for CHEK2 mutations**

The probability that a woman with breast cancer carries an 1100delC CHEK2 mutation is influenced by her age, her family history and her ethnic group. The 1100delC mutation is a founder mutation and has a restricted (albeit widespread) distribution. It is present in white individuals of Northern and Eastern European origin, including descendents of Europeans, such as French-Canadians, Jews and Brazilians (9–21). It does not appear to be present in Southern Europe or in Asia or in most Latin-American populations (20, 22–24). Interestingly, Bell et al. found the 1100delC mutation in three of 345 African-American women with breast cancer, but this association has not been confirmed (17). Therefore, the strongest predictor of the presence of a mutation is ethnic group.

Generally, familial breast cancer appears at an earlier age than non-familial breast cancer. An early age of cancer diagnosis has been associated with the 1100delC mutation in some studies, but not in all, and age at diagnosis should probably not be used as a criterion to exclude women for genetic testing. In the meta-analysis by Weischer et al. (16), the odds ratio for unselected breast cancers was 2.6 and the odds ratio for early-onset breast cancer was 2.7. Women with bilateral breast cancer are more likely to carry a CHEK2 mutation than women with unilateral breast cancer, and women with an ER-positive breast cancer are more likely to carry a mutation than women with ER-negative breast cancer. In the Weischer et al. meta-analysis, between 1% and 6.3% of the familial cases carried a CHEK2 mutation, depending on the country of origin. Countries with a particularly high prevalence of mutations include Finland and the Netherlands (Table 1). The overall odds ratio for familial breast cancer was 4.8 (16). In summary, the possibility of a CHEK2 mutation should be considered in women with breast cancer and a family history of breast cancer (mutation prevalence between 1% and 6%), particularly (but not exclusively) if cancers are early-onset or bilateral.

It is not advisable to test for the CHEK2 mutation in the absence of a personal or family history of breast cancer, given that mutations are rare and the gene penetrance is likely to be low in the absence of a family history. If unselected unaffected women were to be tested for CHEK2 mutations, in many populations, CHEK2 carriers will outnumber carriers of BRCA1 and BRCA2 mutations combined (19). To some, the commercial implementation of ‘personalised medicine’ includes offering a risk assessment tool to the general public which includes screening with a comprehensive mutation panel. For the reasons outlined above, these companies should be discouraged from including the 1100delC CHEK2 mutation.

Once a CHEK2 mutation has been identified in a breast cancer family, it is reasonable to test the
unaffected women in the family. If an unaffected woman carries a \textit{CHEK2} mutation and has a first-degree relative with breast cancer, her lifetime risk of breast cancer is approximately 20–25%. The risk may be higher than this if the woman has multiple affected relatives. If a woman has unilateral breast cancer and a \textit{CHEK2} mutation, her risk of second primary breast cancer is about 1% per year.

In summary, \textit{CHEK2} mutation testing can provide useful information for women with and without cancer. In the context of a family history, a mutation is associated with a lifetime breast cancer risk in excess of 20%. At this level of risk, intervention with intensified surveillance and/or chemoprevention is reasonable. Because of the rarity of the 1100delC mutation, it is probably not appropriate to offer testing for this allele alone, and it is probably not worthwhile to look for other \textit{CHEK2} mutations by full gene sequencing. However, it is reasonable to include a test for \textit{CHEK2} when \textit{BRCA1} and \textit{BRCA2} testing is conducted. In the absence of a known mutation in the family, mutation testing for breast cancer families almost always includes a screen for both \textit{BRCA1} and \textit{BRCA2}, and for practical reasons, the two tests are done in parallel and not in sequence. It is rational to add the single \textit{CHEK2} allele to this panel. If so, then it is expected that this will be positive in about 2% of tested cases in Canada and the US, and the cost of including this single variant should be modest. Of course, this scenario assumes that the tests for mutations in the three genes will be done in a single reference laboratory.

The principal reason for genetic testing should be to evaluate familial breast cancer. However, in the context of a family history of prostate cancer, the presence of a \textit{CHEK2} mutation is also important. If a man has a \textit{CHEK2} mutation and a first-degree relative with prostate cancer, he should be encouraged to undergo annual prostate-specific antigen (PSA) screening. The benefits of screening for prostate cancer among men with prostate cancer have not been evaluated in \textit{CHEK2} carriers and there is limited evidence to support widespread PSA screening in the general population (60, 61). The 1100delC mutation does not appear to predispose to colon cancer (39), and until further data are available, testing for the missense variant I157T is not advised.

\textbf{Future directions}

This review suggests several directions for further study. The full extent of the ethnic and geographic distribution of the I157T missense variant has not been studied in detail, but it is likely to be more widespread than the 1100delC variant. It should be confirmed that the I157T allele predisposes to colon, prostate and breast cancer in a range of populations. In Canada and the USA, it is unlikely that mutations other than 1100delC will be important causes of breast cancer (17), but it is possible that other founder alleles exist in other ethnic groups. These mutations could be sought by re-sequencing samples collected from familial breast cancers within those populations. The prevalence of the 1100delC mutation in populations of African origin should be studied and additional mutation surveys should be conducted in Latin-American countries. The recommendations for MRI and tamoxifen chemoprevention for female \textit{CHEK2} mutation carriers and of PSA screening for male carriers are based on the analysis of limited information and it is important that these interventions be evaluated in prospective observational studies. The observation that \textit{CHEK2} mutations are protective against lung cancer should be confirmed in other studies and the therapeutic implications of this observation should be explored.

\textbf{Acknowledgements}

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\textbf{References}


