Chapter

Genomic profiling of *CHEK2**1100delC-mutated breast carcinomas.

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Abstract

Introduction *CHEK2**1100delC is a moderate-risk breast cancer susceptibility allele with a high prevalence in the Netherlands. We performed copy number and gene expression profiling to investigate whether *CHEK2**1100delC breast cancers harbor characteristic genomic aberrations, as seen for *BRCA1* mutated breast cancers.

Methods We have performed high-resolution SNP array and gene expression profiling of 120 familial breast carcinomas selected from a larger cohort of 155 familial breast tumours, including *BRCA1*, *BRCA2*, and *CHEK2* mutant tumours. Gene expression analyses based on a mRNA immune signature was used to identify samples with relative low amounts of tumour infiltrating lymphocytes, which were previously found to disturb tumour copy number and (loss of heterozygosity) LOH profiling. We specifically compared the genomic and gene expression profiles of *CHEK2**1100delC breast cancers (n=14) with BRCAX (familial non-*BRCA1*/*BRCA2*/*CHEK2**1100delC mutated) breast cancers (n=34) of the luminal intrinsic subtypes for which both SNP-array and gene expression data is available.

Results Based on gene expression analysis, relative high amounts of tumour infiltrating lymphocytes (TILs) were found in a relatively small number of luminal breast cancers as compared to breast cancers of the basal-like subtype. As expected, these samples mostly have very few copy number aberrations and no detectable regions of LOH. By unsupervised hierarchical clustering of copy number data we observed a great degree of heterogeneity amongst the *CHEK2**1100delC breast cancers, comparable to the BRCAX breast cancers. Furthermore, copy number aberrations were mostly seen at low frequencies in both the *CHEK2**1100delC and BRCAX group of breast cancers. However, supervised class comparison identified copy number loss of chromosomal arm 1p possibly associated with *CHEK2**1100delC status.

Conclusions In conclusion, in contrast to basal-like *BRCA1* mutated breast cancers, no apparent specific somatic copy number aberration (CNA) profile for *CHEK2**1100delC breast cancers was found. With the possible exception of copy number loss of chromosomal arm 1p in a subset of tumours, which might be involved in *CHEK2* tumourigenesis. This difference in CNAs profiles might be explained by the need for *BRCA1*-deficient tumour cells to acquire survival factors, by for example specific copy number aberrations, to expand. Such factors may not be needed for breast tumours with a defect in a non-essential gene such as *CHEK2*.

Introduction

Approximately 10-15% percent of all breast cancer cases arise within a familial clustering of multiple breast cancer cases. Inherited germ-line mutations in the high risk genes *BRCA1* or *BRCA2* are identified in approximately 20 percent of these breast cancer families. In addition, mutations in the *CHEK2*, *PALB2*, *ATM* and *BRIP1* genes confer a moderate lifetime risk of breast cancer but are rare and account for less than 5% of familial breast cancer cases [1].

*CHEK2**1100delC is a moderate-risk breast cancer susceptibility allele with a relatively high prevalence in the Netherlands of 1.1% in the general population, 2.5% in unselected breast cancer cases, and 4.9% in familial breast cancer cases. Other mutations in the *CHEK2* gene contributing to breast cancer risk are negligible in the Dutch population. The lifetime risk of breast cancer for a female *CHEK2**1100delC mutation carrier from the general population is 20-25%, increasing to 35-45% in a familial breast cancer setting [2-4].

CHEK2 (*Checkpoint kinase 2*) has been shown to be involved in cell cycle control and DNA damage response. ATM (*Ataxia telangiectasia mutated*) phosphorylates CHEK2 in response to DNA damage, resulting in CHEK2 homodimerization. The resulting active kinase exerts its function through its ability to phosphorylate TP53, CDC25A, CDC25C, PLK and BRCA1 [5]. The function of *CHEK2* is abrogated by the 1100*delC frameshift mutation which causes a premature translation stop in the kinase domain of the protein. Both the mRNA, which is degraded through nonsense-mediated mRNA decay, as well as the resulting truncated protein are highly unstable [6,7]. Very few breast tumours from 1100delC carriers show CHEK2 protein expression although LOH of the wild-type allele is infrequently found [8]. In contrast, LOH of the *BRCA1* gene is frequently reported in *BRCA1*-mutated breast cancers [9, Massink et al.]. Also, *BRCA1* mutated breast tumours are frequently reported to be of the basal-like intrinsic subtype, opposed to breast tumours from *CHEK2* mutation carriers, which are reported to be mostly steroid hormone receptor positive (estrogen/progesterone receptor (ER/PR) positive) [10]. In accordance, gene expression profiling assigns tumours from *CHEK2* mutation carriers to the luminal intrinsic subtypes [11].

We and others have shown specific somatic profiles of copy number aberrations (CNAs) characteristic for both *BRCA1* and *BRCA2*-associated breast carcinomas [12-16, Massink et al.]. These CNAs are thought to reflect specific oncogenic pathways in tumour development. The identification of driver genes in these genomic regions could lead to a better understanding

of the underlying process of tumourigenesis and may provide novel clues for targeted therapies.

In this study we have performed high-resolution copy number, LOH and gene expression profiling of 120 familial breast carcinomas selected from a larger cohort of 155 familial breast tumours, including *BRCA1*, *BRCA2* and *CHEK2* mutant tumours. Samples were selected for low amounts of tumour infiltrating lymphocytes by mRNA profiling because tumour infiltrating lymphocytes (TILs) were previously shown to have detrimental effects on genomic profiling of tumour material [Massink et al.] To ascertain whether *CHEK2**1100delC breast cancers harbor characteristic genomic aberrations, as seen in *BRCA1* mutated breast cancers, we specifically compared the genomic profiles of 14 *CHEK2**1100delC breast cancers and 34 BRCAX (familial non-*BRCA1*/*BRCA2*/*CHEK2**1100delC mutated) breast cancers of the luminal intrinsic subtypes for which both SNP-array and gene expression data is available. We compared our results with previously reported findings on genomic and gene expression profiling of *CHEK2**1100delC breast cancers [17].

Materials and Methods

Ethics Statement

This study has been approved by the medical ethical committee at Erasmus MC, and was performed according the Code of Conduct of the Federation of Medical Scientific Societies in The Netherlands.

Sample collection

Fresh-frozen specimens of primary breast tumours from female familial breast cancer cases were selected from the tissue bank of the Erasmus Medical Center Rotterdam. All cases had been screened for germline mutations in *BRCA1*, *BRCA2* and for the *CHEK2**1100delC mutation. The complete breast cancer cohort consists of 155 primary tumours and includes 26 tumours with a *CHEK2**1100delC mutation, 47 BRCA1-mutated tumours, 6 *BRCA2*-mutated tumours, and 76 non-*BRCA1*/*BRCA2*/*CHEK2**1100delC mutated (BRCAX) tumours. These BRCAX breast cancer cases all originated from families with at least two breast cancer cases in first or second degree relatives of which at least one had been diagnosed before the age of 60. The entire cohort has been described in detail [11, Massink et al.]. In this study, 120 tumour samples for which both SNP array and gene expression data is available were used for further analyses. The gene expression and SNP microarray data have been deposited in

NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number 54219.

Gene expression microarrays

For gene expression analysis .CEL files of the individual samples as deposited in GEO 54219 were used. The data was analyzed in Partek Genomics Suite (v6.6, Partek Inc.). Detection of differential gene expression was performed by ANOVA analysis, genes with FDR-stepup (false discovery rate) p-values less than 0.05 were considered to be statistically significant differentially expressed.

Classification of Intrinsic Molecular Subtypes

The intrinsic gene list was used to appoint the samples to molecular subtypes as described [11]. In short, the intrinsic gene list [18] was mapped to the corresponding probe-sets on the HGU_133_plus_2.0 array using Unigene Cluster Id's. The most variable probe-sets were used to cluster 120 familial samples using average linkage hierarchical clustering with correlation as a distance metric. In this paper, the luminal A and B samples together are referred to as luminal samples.

mRNA based sample selection

To select for samples with relative low number of tumour infiltrating lymphocytes (TILs), hierarchical clustering of expression data was used. This approach has largely been described in our previous work [Massink et al.]. In short, the proportion of lymphocytic nuclei of 96 tumour samples was assessed on H&E-stained frozen sections. For subsequent mRNA analysis, the luminal and basal samples were processed separately. These samples were divided in two groups based on the TIL percentages (high and low TIL count, median split) on which subsequent ANOVA analysis was performed to find differentially expressed probe sets passing a FDR p-value <0.05. Finally, the overlapping probe-sets for the luminal and basal sample sets were determined to create the final mRNA immune signature, Additional File 1. Following this approach, 14 *CHEK2*1100delC* and 34 *BRCAX* breast cancers with relative low levels of this expression signature were selected for further supervised analyses.

Copy number analyses by SNP arrays

For copy number and LOH analyses, .CEL files of the individual samples as deposited in GEO 54219 were used. In total, 120 samples were processed, consisting of 35 *BRCA1*, 5 *BRCA2*, 17 *CHEK2**1100delC mutated and 63 BRCAX tumour samples. The array intensity .CEL files were processed by Partek Genomics Suite using default settings for background correction and summarization, results were corrected for GC-content and fragment length. Unpaired copy number analysis was performed in Partek Genomics Suite, comparing signal Log2 ratios to a custom created reference baseline of 90 female HapMap samples with European ancestry (CEU). The genomic segmentation algorithm was used to detect breakpoint regions and estimate copy number levels with stringent parameters (P<0.0001, >20 markers, signal/noise: 0.45). With an expected normal range of 2± 0.25 copies. Differences between the tumour groups (mutation class) for frequency of copy number aberrations (gained, lost, or unchanged) were calculated by employing a 3 x 2 Fisher's exact test (FE). Resulting p-values were not directly corrected for multiple testing. SNP array, gene and cytogenetic band locations are based on the hg19 Genome build. For unsupervised hierarchical clustering of copy number data the called copy number states (amplification (copy number >6), gain, loss or neutral) of the segmentation data were used as distance metric. Agglomerative clustering was performed on these data by Euclidean distance and Wards method.

LOH calling by detection of allelic imbalance

A segmentation based approach of allelic imbalances was used to identify regions of loss of heterozygosity (LOH). The B-allele frequencies of 120 breast tumour samples were generated in Partek Genomics Suite. Mirrored BAF profiles (mBAF) were used as previously described [19]. The resulting mBAF profiles were segmented in Partek Genomics Suite and LOH calling of segmented regions was done by applying a fixed allelic imbalance threshold of 0.76 and p-value <0.01. The same parameters used in segmentation of the copy number data were applied, except that a window size of 100 SNPs instead of 20 SNPs was used as a minimum number of genomic markers.

Results

Sample selection for low amount of TILs

To select for samples with low number of TILs, hierarchical clustering of expression data of all 120 breast carcinomas based on the mRNA immune signature was used (Figure 1A). Relative high numbers of TILs were predominantly found in basal-like breast cancers. However, 14 BRCAX and 3 *CHEK2**1100delC samples of the luminal breast cancers were also found to have such high mRNA signature values and were not used in supervised class comparison of CNAs and differential gene expression analysis. Figure 1B shows the correlation between immune signature mRNA values and TIL percentages as determined on H&E stained slides (r_s=0.74, p-value< 0.001).

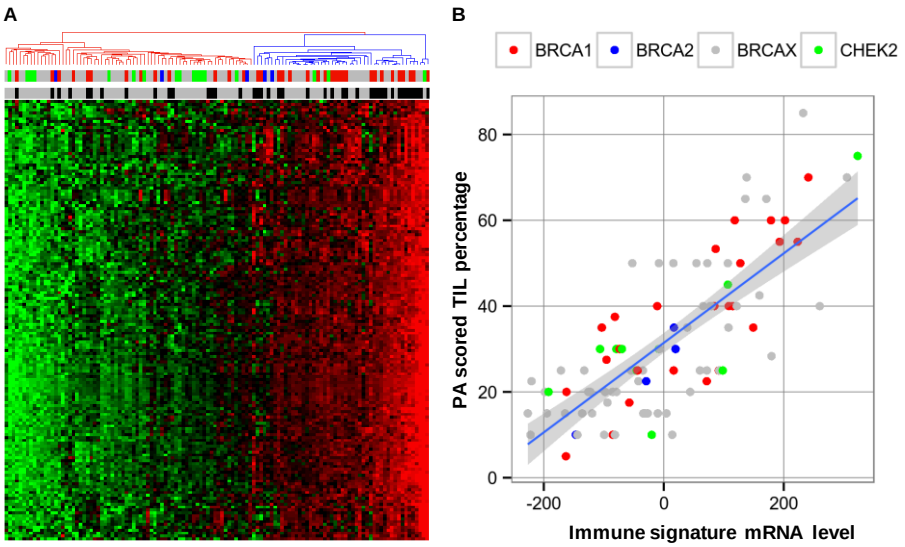

Figure 1. Hierarchical clustering of immune signature mRNA data and correlation with TILs. **A**, hierarchical clustering of mean centred, standardized immune signature gene expression data. Samples in the blue branch are regarded as high TIL (red: relative high expression, green: relative low expression), and are discarded from further analyses. Top row indicates mutation status (red: *BRCA1*, blue: *BRCA2*, green: *CHEK2*, grey: BRCAX), bottom row indicates intrinsic subtype (grey: luminal, black: basal). **B**, correlation plot for immune signature mRNA values and TIL percentages as determined by a pathologist (r_s=0.74, p-value< 0.001), colours represent mutation status.

Copy number and LOH profiling

High resolution copy number and LOH profiling by means of SNP array analysis was performed to gain insight into the genomic characteristics of *CHEK2**1100delC as compared to BRCAX breast cancers. Intrinsic subtyping of breast carcinomas based on global gene expression profiles has revealed large differences between the basal-like, ERBB2/Her2Neu and luminal subtypes regarding patterns of CNAs [12,20,21]. As *CHEK2**1100delC breast cancers are found to be exclusively of the luminal subtypes [11], the analyses were restricted to these intrinsic subtypes to avoid subtype associated confounding effects on copy number profiling.

Unsupervised hierarchical clustering of copy number profiles of all *CHEK2**1100delC and BRCAX samples suggests a great degree of heterogeneity amongst the *CHEK2**1100delC breast cancers comparable to that seen in the BRCAX breast cancers (Figure 2). The copy number aberration clustering roughly divides the samples into three groups. In group 1 many tumours are seen to have similar CNAs, including regions of copy number gain of chromosomal arms 1q, 8q, and 16p and copy number losses of chromosomal arms 8p and 16q. A second group (group 2) of tumours was found to have a more instable CNA profile with more focal amplifications on chromosome 17 (*ERBB2*), while a third group (group 3) is characterized predominantly by high TIL samples with very few CNAs. In agreement with the copy number analysis results, heterogeneous patterns of LOH with no frequent reoccurring regions were identified.

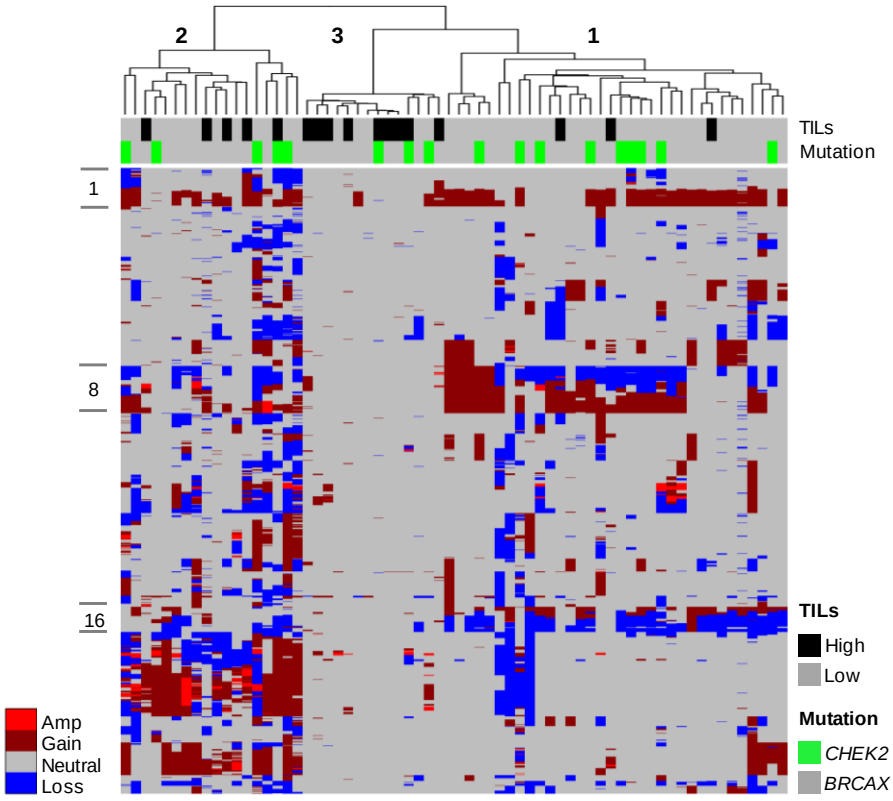

Figure 2. Genomic profiles of *CHEK2**1100delC and BRCAX breast tumours. Hierarchical clustering of CNA data. On the vertical axis, chromosomes 1 to X are displayed. Copy number gains are indicated in dark red (copy number amplifications (copy number > 6) in light red), losses in blue, and copy neutral regions in grey. TIL (black: high TIL, grey: low TIL) and mutation status (green: *CHEK2**1100delC, grey: BRCAX) are indicated for each sample in the top rows by color.

Supervised class comparison of the copy number profiles of low TIL *CHEK2**1100delC (n=14) and BRCAX (n=34) breast cancers identified a small number of genomic regions with differential CNAs (Figure 3). Figure 3A shows the frequency plots for gains and losses observed in the *CHEK2**1100delC and BRCAX breast cancers. Figure 3B shows the Fisher Exact test results on these regions of CNA. Most notably is the copy number loss of chromosome 1p which overlaps with a previously reported region [17]. However, most of the reported regions are marginally statistically significant and have CNAs at very low frequencies in the two tumour groups. Interestingly, a small region of (focal) copy number gain on chromosome 17

(including the *ERBB2* locus) is found in almost half (6/14) of the *CHEK2**1100delC breast cancers.

Copy number losses on chromosome 22 (including the *CHEK2* locus) are found in half of the *CHEK2**1100delC breast cancers, of which 4 showed LOH at the CHEK2 locus. However, copy number losses on chromosome 22 are also frequently found for the BRCAX breast cancers (25% of the cases). Furthermore, samples with copy number losses on chromosome 22 were seen in all 3 groups as identified by unsupervised hierarchical clustering of copy number data.

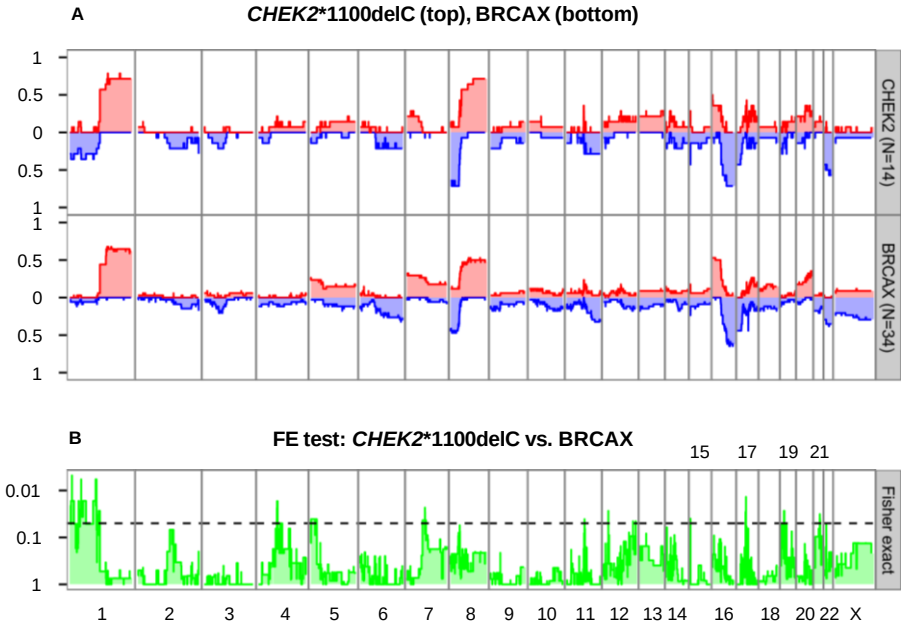

Figure 3. Copy number frequency plots of 14 *CHEK2**1100delC and 34 BRCAX low TIL breast tumours. **A**, the frequency (x-axis) of gains (red) and losses (blue) are displayed along chromosomes 1 to X (y-axis) for 14 *CHEK2**1100delC (top panel) and 34 *BRCAX* (bottom panel) breast cancers. **B**, Fisher's exact test is used to determine regions of differential copy number aberrations between the *CHEK2**1100delC and *BRCAX* breast cancers. The dotted line represents a p-value threshold of 0.05 (not corrected for multiple testing). The regions above the threshold are considered to be significantly different between the groups. P-values are - log 10 transformed.

Gene expression analysis

Gene expression analysis by means of ANOVA was restricted to low TIL samples of the luminal intrinsic subtypes, with mutation status as single factor (14 CHEK2*1100delC vs. 34 BRCAX). This resulted in 6 differentially expressed probe-sets passing a step-up FDR (False-Discovery Rate) p-value of 0.05 (See Table 1). None of the differentially expressed genes between the *CHEK2**1100delC and BRCAX breast cancers were found to overlap with the *CHEK2* signature reported by Muranen et al. [17].

Gene Symbol	Probeset ID	Chromosomal Location	FDR p-value (*CHEK2* vs. BRCAX))	Fold-change (*CHEK2* vs. BRCAX)
NCRNA00201	225786_at	chr1q44	0.00339396	2.4
CENPJ	223513_at	chr13q12.12	0.00339396	1.8
OGT	209240_at	chrXq13	0.0208235	1.5
PRPF4B	202127_at	chr6p25.2	0.0208235	2
PIKFYVE	213111_at	chr2q34	0.0208235	1.5
NFYB	218127_at	chr12q22-q23	0.0284964	1.58

Table 1. Differentially expressed probe-sets between *CHEK2**1100delC and BRCAX breast cancers.

Discussion

We performed high-resolution copy number, LOH and gene expression profiling of *CHEK2**1100delC breast cancers to better understand the tumourigenesis to which the germline *CHEK2* deficiency predisposes. Our analysis was restricted to breast cancers of the luminal intrinsic subtypes as *CHEK2**1100delC breast cancers are found to be exclusively of these subtypes. Furthermore, samples were selected for low numbers of TILs based on a mRNA immune signature.

The copy number profiles of *CHEK2**1100delC breast carcinomas were found to be heterogeneous and largely resemble those of the BRCAX breast carcinomas. The largest group of tumours have characteristic copy number gains of chromosomal arms 1q, 8q and 16p and copy number losses of chromosomal arms 8p and 16q. These CNAs have frequently been reported for breast carcinomas of the luminal intrinsic subtypes [12,21-23]. A second group was found to have a more instable CNA profile and frequent

focal amplifications of the *ERBB2* genomic region. A third group was found to be largely CNA devoid, most of these samples were identified to have high numbers of tumour infiltrating lymphocytes (TILs). In previous work we identified a similar CNA devoid group of (*BRCA1*-mutated) basal-like breast carcinomas, which proved to be caused by the presence of large numbers of TILs in these samples.

Compared to the *BRCA1* and *BRCA2* profiles reported in literature and our previous study, copy number aberrations are infrequently seen in *CHEK2**1100delC breast cancers. The most frequently observed aberrations in *CHEK2**1100delC breast cancers are those seen in many breast cancers of the luminal intrinsic subtypes. Also, hierarchical clustering showed a great degree of heterogeneity of copy number profiles amongst the *CHEK2**1100delC breast cancers, while *BRCA1*-mutated breast cancers frequently co-cluster in hierarchical cluster analysis [12, Massink et al.].

Few characteristic CNAs for *CHEK2**1100delC breast cancers were found. Furthermore, in contrast to our *BRCA1* profiling results [Massink], most of these CNAs in *CHEK2**1100delC breast cancers are seen at very low frequencies and are marginally statistically significant. However, copy number loss of chromosomal arm 1p was found more frequently in *CHEK2**1100delC breast cancers. Furthermore, the frequently observed amplification of the *ERBB2* gene in the *CHEK2**1100delC samples is in line with previous reported results regarding breast tumour characteristics of *CHEK2**1100delC homozygous carriers, in which over-expression of the *ERBB2* gene was found in roughly 50% of the cases in comparison to the known 20-25% in the general breast cancer population [24].

For the most part, our findings are comparable to previously reported findings on the genomic characteristics of *CHEK2**1100delC breast cancers by Muranen et al. [17]. This includes the copy number loss of chromosomal arm 1p seen in *CHEK2**1100delC breast cancers, and LOH/loss at the *CHEK2* locus in only part of the tumours. However, the reported copy number gains of the *CHEK2* region in *CHEK2**1100delC breast cancers were not observed in our data, we only observed normal copy number and copy number losses.

In addition, genome wide gene expression analysis identified a small number of genes to be differentially expressed between *CHEK2**1100delC and BRCAX breast cancers, of which none overlap with the previously reported *CHEK2* gene expression signature by Muranen et al [17]. Furthermore, only 2 genes are seen to overlap with a reported 40-gene *CHEK2* signature found in the study by Nagel et al. [11]. This difference is most likely due to sample selection criteria. Where our analysis is restricted

to low-TIL BRCAX and *CHEK2**1100delC mutated breast cancers of the luminal subtypes, Nagel et al. performed their analysis on all hormone receptor positive breast cancers, including high-TIL and *BRCA1*/*BRCA2* mutated samples. Furthermore, we applied a stringent false discovery p_value cut-off of 0.05, opposed to a FDR p_value of 0.25 by Nagel et al. Also, there is no overlap in the *CHEK2* gene signatures reported by Muranen et al. and Nagel et al.

The most significant differentially expressed gene in the 40-gene *CHEK2* signature is the *CHEK2* gene itself. We found *CHEK2* gene expression to be particularly high in the high-TIL BRCAX samples, this could explain why, after sample selection, we do not find the *CHEK2* gene to be differentially expressed.

All differentially expressed genes in our analysis were found to be relatively higher expressed in the *CHEK2**1100delC samples as compared to the BRCAX samples, but were not found in genomic regions of copy number gain in the *CHEK2**1100delC samples. We found no direct links between these differentially expressed genes and *CHEK2* gene function, except for possibly the *CENPJ* gene. This gene encodes a protein that belongs to the centromere protein family. The protein plays a structural role in the maintenance of centrosome integrity and normal spindle morphology [25]. Recently, *CHEK2* has been reported to be involved in the regulation of proper mitotic spindle formation through phosphorylation of BRCA1, hereby ensuring chromosomal stability [26].

Conclusions

In conclusion, in contrast to *BRCA1/2* breast cancers, no apparent specific copy number aberration (CNA) profile for *CHEK2**1100delC breast cancers was found. This could in part result from the small number of *CHEK2**1100delC breast cancer samples used in this study. However, in our previous work, an even smaller number of *BRCA1*-mutated basal-like breast cancers proved sufficient to identify *BRCA1*-associated CNAs.

The results show no specific tumourigenic events regarding *CHEK2* tumourigenesis, except for copy number loss of chromosomal arm 1p. However, gene expression analysis did not hint towards any potential driver genes in this region. Further studies are needed to establish whether this loss is indeed associated with *CHEK2* breast tumours. Also, gene expression analysis identified a very small number of differentially expressed genes between the *CHEK2**1100delC and BRCAX breast cancers, of which, except for possibly *CENPJ*, none seem to have a putative role in *CHEK2*

related tumourigenesis based on what is known in literature. This small number of differentially expressed genes can also, in part, be due to the small amount of *CHEK2**1100delC samples used in the analysis, and need to be validated.

For *BRCA1* mutated breast cancers, specific CNAs are reported. Complete loss of *BRCA1* leads to severe proliferation defects in normal cells, proving lethal during embryonic development [27]. Therefore, it seems likely that *BRCA1*-mutated cells acquire survival factors that allow *BRCA1*-deficient tumour cells to expand. CNAs are well known mechanisms to acquire such factors. For instance, in previous work we identified a region of copy number loss on chromosome 15q in all *BRCA1*-mutated samples, which we now speculate to act on the reported *BRCA1* associated loss of *53BP1* [25]. In contrast to *BRCA1*, *CHEK2* deficiency is not lethal as evidenced by *CHEK2**1100delC homozygous carriers in the population and the viability of Chek2 knockout mice [28,29]. Therefore, specific survival factors for *CHEK2* mutated cells are not required during tumourigenesis if the wild type allele for *CHEK2* is lost. However, it remains unclear to what extent loss of the wild type allele of *CHEK2* is necessary for tumourigenesis. Although *CHEK2**1100delC homozygous female carriers are more susceptible to tumour development [24], analysis of tumours from *CHEK2**1100delC heterozygous carriers show a heterogeneous pattern of LOH/loss at the *CHEK2* locus. It remains uncertain whether this reflects two different tumour groups, i.e. one with complete loss of wild type *CHEK2* and thereby *CHEK2* driven tumourigenesis and one without thereby representing sporadic tumours, or that loss of one *CHEK2* allele is sufficient to drive tumourigenesis.

Based on our previous work on *BRCA1* tumours and the current study on *CHEK2* tumours we postulate a model in which breast tumours with a defect in an essential gene such as *BRCA1* or *BRCA2*, result in copy number profiles that reflect both the tumour subtype and specific surviving factors while breast tumours with a defect in a non-essential gene such as *CHEK2*, result in copy number profiles that largely reflect the tumour subtype. In this model the presence of a germline *CHEK2**1100delC mutation might be regarded as an accelerator of tumourigenesis leading to CNA profiles comparable to that of luminal sporadic breast tumours.

References

1. Ellsworth RE, Decewicz DJ, Shriver CD, Ellsworth DL: **Breast cancer in the personal genomics era.** *Curr Genomics* 2010, **11:**146-161.

2. Meijers-Heijboer H, van den OA, Klijn J, Wasielewski M, de SA, Oldenburg R, Hollestelle A, Houben M, Crepin E, van Veghel-Plandsoen M et al.: **Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations.** *Nat Genet* 2002, **31:**55-59.

3. **CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies.** *Am J Hum Genet* 2004, **74:**1175-1182.

4. Cybulski C, Wokolorczyk D, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Masojc B, Deebniak T, Gorski B, Blecharz P et al.: **Risk of breast cancer in women with a CHEK2 mutation with and without a family history of breast cancer.** *J Clin Oncol* 2011, **29:**3747-3752.

5. Bartek J, Lukas J: **Chk1 and Chk2 kinases in checkpoint control and cancer.** *Cancer Cell* 2003, **3:**421-429.

6. Staalesen V, Falck J, Geisler S, Bartkova J, Borresen-Dale AL, Lukas J, Lillehaug JR, Bartek J, Lonning PE: **Alternative splicing and mutation status of CHEK2 in stage III breast cancer.** *Oncogene* 2004, **23:**8535-8544.

7. Anczukow O, Ware MD, Buisson M, Zetoune AB, Stoppa-Lyonnet D, Sinilnikova OM, Mazoyer S: **Does the nonsense-mediated mRNA decay mechanism prevent the synthesis of truncated BRCA1, CHK2, and p53 proteins?** *Hum Mutat* 2008, **29:**65-73.

8. Oldenburg RA, Kroeze-Jansema K, Kraan J, Morreau H, Klijn JG, Hoogerbrugge N, Ligtenberg MJ, van Asperen CJ, Vasen HF, Meijers C et al.: **The CHEK2*1100delC variant acts as a breast cancer risk modifier in non-BRCA1/BRCA2 multiple-case families.** *Cancer Res* 2003, **63:**8153-8157.

9. Merajver SD, Frank TS, Xu J, Pham TM, Calzone KA, nett-Baker P, Chamberlain J, Boyd J, Garber JE, Collins FS et al.: **Germline BRCA1 mutations and loss of the wild-type allele in tumours from families with early onset breast and ovarian cancer.** *Clin Cancer Res* 1995, **1:**539-544.

10. de Bock GH, Schutte M, Krol-Warmerdam EM, Seynaeve C, Blom J, Brekelmans CT, Meijers-Heijboer H, van Asperen CJ, Cornelisse CJ, Devilee P et al.: **Tumour characteristics and prognosis of breast cancer patients carrying the germline CHEK2*1100delC variant.** *J Med Genet* 2004, **41:**731-735.

11. Nagel JH, Peeters JK, Smid M, Sieuwerts AM, Wasielewski M, de W, V, Trapman-Jansen AM, van den OA, Bruggenwirth H, van IJW et al.: **Gene expression profiling assigns CHEK2 1100delC breast cancers to the luminal intrinsic subtypes.** *Breast Cancer Res Treat* 2012, **132:**439-448.

12. Jonsson G, Staaf J, Vallon-Christersson J, Ringner M, Holm K, Hegardt C, Gunnarsson H, Fagerholm R, Strand C, Agnarsson BA et al.: **Genomic subtypes of breast cancer identified by array-comparative genomic hybridization display distinct molecular and clinical characteristics.** *Breast Cancer Res* 2010, **12:**R42.

13. Tirkkonen M, Johannsson O, Agnarsson BA, Olsson H, Ingvarsson S, Karhu R, Tanner M, Isola J, Barkardottir RB, Borg A et al.: **Distinct somatic genetic changes associated with tumour progression in carriers of BRCA1 and BRCA2 germ-line mutations.** *Cancer Res* 1997, **57:**1222-1227.

14. Joosse SA, Brandwijk KI, Devilee P, Wesseling J, Hogervorst FB, Verhoef S, Nederlof PM: **Prediction of BRCA2-association in hereditary breast carcinomas using array-CGH.** *Breast Cancer Res Treat* 2010.

15. van Beers EH, van WT, Wessels LF, Li Y, Oldenburg RA, Devilee P, Cornelisse CJ, Verhoef S, Hogervorst FB, van't Veer LJ et al.: **Comparative genomic hybridization profiles in human BRCA1 and BRCA2 breast tumours highlight differential sets of genomic aberrations.** *Cancer Res* 2005, **65:**822-827.

16. Wessels LF, van WT, Hart AA, van't Veer LJ, Reinders MJ, Nederlof PM: **Molecular classification of breast carcinomas by comparative genomic hybridization: a specific somatic genetic profile for BRCA1 tumours.** *Cancer Res* 2002, **62:**7110-7117.

17. Muranen TA, Greco D, Fagerholm R, Kilpivaara O, Kampjarvi K, Aittomaki K, Blomqvist C, Heikkila P, Borg A, Nevanlinna H: **Breast tumours from CHEK2 1100delC-mutation carriers: genomic landscape and clinical implications.** *Breast Cancer Res* 2011, **13:**R90.

18. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S et al.: **Repeated observation of breast tumour subtypes in independent gene expression data sets.** *Proc Natl Acad Sci U S A* 2003, **100:**8418-8423.

19. Staaf J, Lindgren D, Vallon-Christersson J, Isaksson A, Goransson H, Juliusson G, Rosenquist R, Hoglund M, Borg A, Ringner M: **Segmentation-based detection of allelic imbalance and loss-of-heterozygosity in cancer cells using whole genome SNP arrays.** *Genome Biol* 2008, **9:**R136.

20. Smid M, Hoes M, Sieuwerts AM, Sleijfer S, Zhang Y, Wang Y, Foekens JA, Martens JW: **Patterns and incidence of chromosomal instability and their prognostic relevance in breast cancer subtypes.** *Breast Cancer Res Treat* 2011, **128:**23-30.

21. Waddell N, Arnold J, Cocciardi S, da SL, Marsh A, Riley J, Johnstone CN, Orloff M, Assie G, Eng C et al.: **Subtypes of familial breast tumours revealed by expression and copy number profiling.** *Breast Cancer Res Treat* 2010, **123:**661-677.

22. Smid M, Hoes M, Sieuwerts AM, Sleijfer S, Zhang Y, Wang Y, Foekens JA, Martens JW: **Patterns and incidence of chromosomal instability and their prognostic relevance in breast cancer subtypes.** *Breast Cancer Res Treat* 2010, **128:**23-30.

23. Bergamaschi A, Kim YH, Wang P, Sorlie T, Hernandez-Boussard T, Lonning PE, Tibshirani R, Borresen-Dale AL, Pollack JR: **Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer.** *Genes Chromosomes Cancer* 2006, **45:**1033-1040.

24. Adank MA, Jonker MA, Kluijt I, van Mil SE, Oldenburg RA, Mooi WJ, Hogervorst FB, van den Ouweland AM, Gille JJ, Schmidt MK et al.: **CHEK2*1100delC homozygosity is associated with a high breast cancer risk in women.** *J Med Genet* 2011, **48:**860-863.

25. Lin YC, Chang CW, Hsu WB, Tang CJ, Lin YN, Chou EJ, Wu CT, Tang TK: **Human microcephaly protein CEP135 binds to hSAS-6 and CPAP, and is required for centriole assembly.** *EMBO J* 2013, **32:**1141-1154.

26. Stolz A, Ertych N, Kienitz A, Vogel C, Schneider V, Fritz B, Jacob R, Dittmar G, Weichert W, Petersen I et al.: **The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells.** *Nat Cell Biol* 2010, **12:**492-499.

27. Evers B, Jonkers J: **Mouse models of BRCA1 and BRCA2 deficiency: past lessons, current understanding and future prospects.** *Oncogene* 2006, **25:**5885-5897.

28. Hirao A, Cheung A, Duncan G, Girard PM, Elia AJ, Wakeham A, Okada H, Sarkissian T, Wong JA, Sakai T et al.: **Chk2 is a tumour suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner.** *Mol Cell Biol* 2002, **22:**6521-6532.

29. Takai H, Naka K, Okada Y, Watanabe M, Harada N, Saito S, Anderson CW, Appella E, Nakanishi M, Suzuki H et al.: **Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription.** *EMBO J* 2002, **21:**5195-5205.