A Genome-Wide Association Study to Identify Potential Germline Copy Number Variants for Sporadic Breast Cancer Susceptibility

Yadav Sapkota^{a, b} Ashok Narasimhan^c Mahalakshmi Kumaran^c
Badan S. Sehrawat^c Sambasivarao Damaraju^{c, d}

^aDepartment of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, Tenn., USA;

^bStatistical Genetics Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Qld., Australia; ^cDepartment of Laboratory Medicine and Pathology, University of Alberta, and ^dCross Cancer Institute, Alberta Health Services, Edmonton, Alta., Canada

Key Words

Association study · *BRCA* · Breast cancer susceptibility · Copy number variations · Copy number variation calling algorithm · Genetic predisposition · Germline DNA · Polygenic disease · Single nucleotide polymorphism · Sporadic breast cancer

Abstract

Breast cancer (BC) predisposition in populations arises from both genetic and nongenetic risk factors. Structural variations such as copy number variations (CNVs) are heritable determinants for disease susceptibility. The primary objectives of this study are (1) to identify CNVs associated with sporadic BC using a genome-wide association study (GWAS) design; (2) to utilize 2 distinct CNV calling algorithms to identify concordant CNVs as a strategy to reduce false positive associations in the hypothesis-generating GWAS discovery phase, and (3) to identify potential candidate CNVs for follow-up replication studies. We used Affymetrix SNP Array 6.0 data profiled on Caucasian subjects (422 cases/348 controls) to call CNVs using algorithms implemented in Nexus Copy Number and Partek Genomics Suite software. Nexus algorithm identified CNVs associated with BC (731 autosomal CNVs with >5% frequency in the total sample and $Q < 0.05$). Thirteen CNVs were identified when Partek algorithm-called

CNVs were overlapped with Nexus-identified CNVs; these CNVs showed concordances for frequency, effect size, and direction. Coding genes present within BC-associated CNVs were known to play a role in disease etiology and prognosis. Long noncoding RNAs identified within CNVs showed tissue-specific expression, indicating potential functional relevance of the findings. The identified candidate CNVs warrant independent replication.

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Breast cancer (BC) is the most frequently diagnosed cancer among women worldwide, with more than a million new cases each year. The disease results from the combined effects of genetic, environmental, reproductive, and lifestyle risk factors [Sehrawat et al., 2011; Sapkota et al., 2013a, b]. Single nucleotide polymorphisms (SNPs) identified through genome-wide association studies (GWAS) helped explain only a small proportion of genetic risk in BC [Stacey et al., 2007, 2008; Kidd et al., 2008; Krepischi et al., 2012a; Kuusisto et al., 2013; Long et al., 2013; Michailidou et al., 2013; Sapkota et al., 2013b, 2014]. It is believed that additional variants need to be

Y.S. and A.N. contributed equally to this work.

identified to account for the ‘missing heritability’ [Michailidou et al., 2013]. In addition to SNPs as heritable markers, variations in the germline DNA such as insertions, deletions, and copy number variations (CNVs) are hypothesized to confer genetic susceptibility to complex traits [Feuk et al., 2006; Conrad and Hurler, 2007; McCarroll and Altshuler, 2007; Bailey et al., 2008; Diskin et al., 2009; Conrad et al., 2010b; McCarroll, 2010; Wellcome Trust Case Control Consortium et al., 2010; Masson et al., 2014; Handsaker et al., 2015]. Germline CNVs, therefore, are pursued as potential candidates to explain the missing heritability. This optimism stems from the fact that CNVs occupy a larger portion of the genome compared to SNPs. CNVs are the most common type of structural variations in the human genome and are generally defined as gains or losses of DNA segments ≥ 1 kb. Currently, these cut-offs have been redefined to include microdeletions and amplifications [Conrad and Hurler, 2007; McCarroll and Altshuler, 2007; Conrad et al., 2010a; McCarroll, 2010]. CNVs may exert their phenotypic effects through either gene dosage or *cis*-acting regulatory elements. Over 400,000 unique CNVs were reported in the Database of Genomic Variants (DGV; <http://dgv.tcag.ca/dgv/app/home>) as of 2014 [MacDonald et al., 2014].

Recently, rare CNVs were shown to be important sources for interindividual variations in the susceptibility to familial BC [Gonzalez et al., 2005; Krepischi et al., 2012b; Pylkas et al., 2012; Kuusisto et al., 2013; Masson et al., 2014; Park et al., 2015]. Further, we have demonstrated that copy-neutral losses of heterozygosity are also an important class of structural variations and serve as biomarkers of prognostic value in a BC setting [Sapkota et al., 2013a]. Large CNVs have an appreciable impact on many human diseases, including non-small-cell lung cancer, HIV infection, developmental delay, schizophrenia, autism, and idiopathic learning disability [Glessner et al., 2009, 2010; Hollox and Hoh, 2014; Li et al., 2014]. However, data on the role of structural variations in predisposition to sporadic BC susceptibility is scanty.

Herein, we sought to investigate the role of germline CNVs in sporadic BC susceptibility using 422 invasive BC cases and 348 healthy individuals from Alberta, Canada, which were genotyped employing Affymetrix Genome-Wide Human SNP Array 6.0 [Sapkota et al., 2013a]. Currently, there are no consensus guiding principles on the use of segmentation algorithms, nor ideal SNP or CNV arrays from any manufacturer to identify CNVs associated with disease susceptibility. The catalogues of variants available as a source of reference are of limited utility, and a gold standard reference is still evasive, contrary to the

previous attempts to create such a reference database [Kidd et al., 2008; Korn et al., 2008; Conrad et al., 2010b; Zhang et al., 2011]. High-throughput methods are not available to validate these signatures in a cost-effective manner, and qRT-PCR is one suggested approach to select a number of targets for a limited sample size [Lin et al., 2011]. Keeping these limitations in mind, several recent studies made recommendations to use more than 1 CNV calling algorithm to ascertain CNV calls [Lin et al., 2011; Zhang et al., 2011]. We therefore designed our study to include 2 CNV calling algorithms, Partek Genomics Suite 6.6 (Partek GS) and Nexus Copy Number 6.0 (Nexus CN), respectively. The rationale for the use of 2 algorithms was to overcome inherent constraints by the individual algorithms in ascertaining the breakpoints, an otherwise tedious task, and to enable identification of overlapping CNVs [Conrad et al., 2010a]. Our emphasis is also on identifying common CNVs as heritability determinants. Common CNVs are defined as those which are present in frequencies of $>5\%$ in the total cohort (cases and controls together).

The comparisons between algorithms potentially identify concordant signatures. Concordance criteria are defined as CNVs with (1) 100% region overlap between Nexus and Partek, (2) similar copy number status, and (3) effect size in a similar direction (gain or loss). To our knowledge, this is the first study in CNV-GWAS for BC to apply stringent filtering criteria of 100% concordance by the above selected criteria. Thus, the present study offers a novel premise hitherto unexplored to identify common CNVs as genetic susceptibility determinants in BC. Exploring the possible functional significance of the identified CNVs may offer insights into the etiology of the disease and provide potential loci for replication in independent studies.

Materials and Methods

Study Participants

Cases ($n = 422$) included in this study were predominantly of sporadic BC (defined by age of onset), non-metastatic at the time of diagnosis, and were accessed from the provincial tumor bank, the Alberta Cancer Research Biobank (formerly Canadian Breast Cancer Foundation, CBCF, Tumor Bank) located at the Cross Cancer Institute, Edmonton, Alta., Canada (<http://www.acrb.ca/about-us/>). The tumor bank database also provided clinicopathological information of the stored samples. A detailed description of the bank and the specimens used in this study has been given elsewhere [Sehrawat et al., 2011; Sapkota et al., 2013a, b]. The cases had a pathologically confirmed diagnosis of invasive BC and were predominantly characterized by the late onset of the disease ($>90\%$ of the cases were aged 40+ years at the time of diagnosis;

table 1). The controls (n = 348) used in this study were healthy women (cancer free with no documented family history of cancer at the time of recruitment) from the same geographic location (Alberta, Canada). The controls were accessed from the Tomorrow Project (<http://in4tomorrow.ca>), a prospective cohort study which aims to collect DNA and information about health and lifestyle factors of ~50,000 healthy Albertans to understand the etiology of complex diseases, including cancers and cardiovascular diseases [Sehrawat et al., 2011; Sapkota et al., 2013a, b]. The controls were approximately frequency matched to cases based on a 10-year age group, with >86% of the controls aged 40+ at the time of recruitment in the study. Both cases and controls were predominantly of Caucasian origin.

DNA Extraction and Genotyping

Germline DNA was extracted from peripheral blood samples of both cases and controls, using commercially available Qiagen™ (Mississauga, Ont., Canada) DNA isolation kits, as described earlier [Sehrawat et al., 2011; Sapkota et al., 2013a]. Whole genome genotyping was carried out using the Affymetrix Genome-Wide Human SNP Array 6.0, following the guidelines provided by the manufacturer. CNV signal intensity data was acquired directly from 'CEL' files by using data import functionality of Partek GS and Nexus CN. Genotype data was acquired by using a default Affymetrix genotyping algorithm (Birdseed v2). Sample qualities were assessed by contrast quality control (CQC), a measure of performance of genotyping experiments recommended by the manufacturer. All samples had CQC >2.0; a CQC ≥1.7 is considered acceptable [Sehrawat et al., 2011].

Population Stratification

We used the principal component analysis-based Eigenstrat method to control for potential confounding effects of population stratification, using SNP genotype data of the 770 samples (cases and controls), as described earlier [Sehrawat et al., 2011; Sapkota et al., 2013a]. Publicly available genotype data of the 270 HapMap samples was used as a reference to infer the genetic ancestry of the samples. We detected 84 individuals (56 cases and 28 controls) as non-Europeans, using ≥3 standard deviations from the top 2 principal components (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000448558). These ancestry outliers were removed, leaving 366 cases and 320 controls for the downstream analysis. Cases and controls employed in the study showed no cryptic relatedness and were detected by assessing the identity by descent (IBD) estimates; all pair-wise IBD correlations were <0.25, as described earlier [Purcell et al., 2007].

Workflow for Identification and Analysis of CNVs Using Nexus CN 6.0

Intensity files of the 270 HapMap samples were used as a reference/baseline to calculate log₂ ratios and B-allele frequencies for each sample, followed by quantile normalization. Probe-to-probe variance was calculated and used to identify extreme outliers due to copy number breakpoints. Using a default value of 3% for outlier removal, which is 1.5% of probes each on the top and bottom of the list of total probes, we removed 6 cases showing higher CQC scores vis-à-vis elevated noise to signal ratios, leaving 360 cases and 320 controls (total n = 680) for the final analysis. SNP-Fast Adaptation Segmentation Technique 2 (SNP-FASST2) segmentation algorithm, combined with quadratic wave correction imple-

Table 1. Patient characteristics

Characteristics	BC cases (n = 422)	Healthy controls (n = 348)
Age group ^a		
<40 years	39	40
40–50 years	129	130
50–60 years	153	99
60–70 years	64	77
70–80 years	29	2
>80 years	8	0
Median age, years	52	50
Range	21–90	36–70
BMI group		
<18.5	2	4
18.5–24.99	121	155
25.0–39.99	233	177
>40	23	12
Missing	43	0
Median BMI	27.4	25.3
25–75th percentile	24.3–31.4	22.9–29.8

^a Age was recorded at diagnosis (for cases) and at blood withdrawal (for controls).

mented in Nexus was used to identify CNVs in the 680 samples. The SNP-FASST2 is a Hidden Markov Model-based segmentation algorithm, which uses normalized log₂ ratio values of ~1.8 million SNP and copy number probes in the Affymetrix Genome-Wide Human SNP Array 6.0 to make a CNV call. The statistical significance threshold for a copy number call was set at 5.0×10^{-10} , with a minimum number of probes per segment of 25 and maximum contiguous probe spacing of 5 kb. Log₂ ratio values of 0.2 and –0.2 were used to define single copy number gains and losses, respectively, while log₂ ratio values of 0.7 and –1.1 were used to define more than 2 copies of gains and losses, respectively. Auto gender correction was applied to call CNVs in X chromosomes.

Workflow for CNV Analysis Using Partek Genomics Suite 6.6

Intensity data from Affymetrix CEL files (n = 686) was imported into Partek GS and quantile normalized. Copy number segments were identified using the genomic segmentation algorithm with the following default settings: minimum number of probes per segment >10 (referred to as genomic markers by Partek); p value threshold = 0.001; signal/noise ratio = 0.3; diploid copy number = 1.7–2.3, with copy number loss and gain below and above these thresholds, respectively. The segmentation data obtained was processed using 270 HapMap samples to create the baseline (representing copy neutral state for a diploid genome) as described above for the Nexus platform. Genome assembly from Genome Reference Consortium human (GRCh) build 37 (Human Genome version, hg19) was used as a source to annotate the identified genomic variants. The identified CNVs were also interrogated in DGV, and the percent overlaps of the CNVs with the recent version of DGV were reported (<http://dgv.tcag.ca/>).

Fig. 1. Size distribution of CNVRs from Partek signatures relative to Nexus CNVRs.

Since the 2 algorithms we used implement their own sample pre-processing and analysis steps, and there is no easier way to standardize these steps for absolute comparisons of algorithm efficiency in identifying the CNVs, the default parameters recommended were used. We therefore made a systematic effort to compare signatures based on criteria of concordances of CNV calls between algorithms (described above). Previous investigators used an arbitrary cut-off of 25–50% of overlap of the sequence as a criterion for concordance [Lin et al., 2011; Zhang et al., 2011; Castellani et al., 2014]. However, we defined concordance as 100% overlap of CNV regions to be on the more stringent side to accommodate the mismatch of default parameters for segmentation by individual algorithms. The deviations from less than 100% of the region overlap, if considered (and as reported by others), would only result in a higher concordance and not less. However, we refrained from such assumptions, and as such many of the identified CNVs in our study or their potential concordances may have been underestimated in our analysis. This is true of any algorithm comparisons implemented thus far for CNV association analysis, as acknowledged by others [Grayson and Aune, 2011; Lin et al., 2011; Zhang et al., 2011; Cantsilieris et al., 2014; Castellani et al., 2014].

Association of CNVs with BC Susceptibility

Frequencies of germline CNVs were compared between cases and controls using 2-tailed Fisher's exact test (within Nexus CN) or Pearson's 2×2 χ^2 test (within Partek GS) to identify CNVs associated ($Q < 0.05$) with BC susceptibility. The 2×2 contingency table compares the frequency of copy number status (loss or gain) between the cases and controls. Correction for multiple comparisons was carried out using the Benjamini-Hochberg false discovery rate and given as a Q value. We report associations with deletions only or gains only from gene and non-gene regions in a case-control setting. We used the 'gap' package [Szatkiewicz et al., 2014] using the R program (<https://cran.rproject.org/web/packages/gap/vignettes/gap.pdf>) to calculate the power needed to detect CNVs associated with BC susceptibility under the following assumptions: additive model, sample size $n = 770$, and life-time risk for BC at 11% (1/9 among Caucasians). For a genotype relative risk of 1.5

and a risk allele frequency range of 5–10%, the power is 32–52%; for a relative risk of 2, the estimated power at risk allele frequency of 5–10% is 77–94%.

Functional Relevance of the BC-Associated CNVs

BC-associated CNVs may harbor *cis*-acting elements and potentially regulate the expression of flanking genes [Wu et al., 2012]. Similarly, CNVs may also harbor non-protein-coding genes such as microRNAs (miRNAs), long noncoding RNAs (lncRNAs), etc. We interrogated for miRNA genes embedded within CNV regions (<https://genome.ucsc.edu/>) and lncRNAs using GENCODE database (<http://www.genencodegenes.org/>) [Meng et al., 2014; Sorensen et al., 2015]. We used TANRIC (http://ibl.mdanderson.org/tanric/_design/basic/index.html) to gain potential insights into the breast tissue-specific expression of lncRNAs [Li et al., 2015]. TANRIC uses The Cancer Genome Atlas (TCGA) transcriptome data generated on the next-generation sequencing platform from as many as 20 different tumor types including BC.

Results

Characteristics of the Identified Germline CNVs

We identified 13,417 CNVs (~50% losses and ~50% gains) in Nexus, of which 731 were autosomal CNVs showing association with BC ($Q < 0.05$) at frequencies of >5% in the sample cohort (online suppl. table 1). We identified 20,154 CNVs (~68% losses and 32% gains) using the Partek GS algorithm, of which 7,155 were autosomal and showed association with BC ($p < 0.05$; data not shown). The CNVs identified by each algorithm had consistent patterns in terms of their mean length across all autosomes. There were no large deviations observed within the algorithm for the mean length of CNVs across

all chromosomes (online suppl. fig. 2A). However, a comparison of the CNVs identified between independent algorithms (from Nexus CN and Partek GS) revealed several distinct characteristics. The Nexus CN-identified CNVs were larger (mean = 35.86 kb) compared to the Partek GS signatures (mean = 3.88 kb), and these differences in their mean size are statistically significant (t test, $p < 0.001$) (online suppl. fig. 2A). Also, the numbers of CNVs identified by the individual algorithms were different as also the size range for the identified CNVs (online suppl. fig. 2B). The CNVs identified by Partek GS showed regions of contiguity with flanking CNVs and as such were merged and designated as copy number variable regions (CNVRs). We compared the CNVRs from Partek GS with Nexus CN CNVs (fig. 1). Partek GS-identified CNVRs were in the range of 1–50 kb, whereas Nexus CNVs were in the range of 1 to >90 kb. Because of these differences and owing to the predominantly large size of CNVs identified by Nexus, in our subsequent experiments we mapped the Partek GS signatures to Nexus CN to show concordance of CNVs identified by both algorithms.

Concordant CNVs Identified by Nexus CN and Partek GS Algorithms

We investigated if the CNVs identified by Nexus CN and Partek GS algorithms would show concordance. The concordant signatures were defined as CNVs with (1) 100% region overlap between Nexus and Partek, (2) similar copy number status, and (3) effect size in a similar direction. Data in table 2 indicates that 13 of the Nexus CN-identified CNVs showed concordance with Partek GS signatures. Of these, 6 CNVs harbored protein-coding genes, and the rest were in intergenic regions. Overall, the CNVs identified were from 8 distinct chromosomes. The CNV length for concordant signatures ranged from 6.7 to 41.7 kb, and showed a differential frequency between cases and controls in the range of 5–13.75. The concordant CNVs appear to be independent disease risk loci as we did not observe linkage disequilibrium with GWAS-identified SNP loci for BC susceptibility (data not shown).

The odds ratios, i.e., effect sizes for CNVs associated with BC risk were higher (Winner's curse!) [Thompson et al., 2011; Bush and Moore, 2012]; the higher odds ratios, if confirmed in independent cohorts, may support the general observations that CNVs associated with phenotypes confer a higher effect size than the SNP-based associations [Lin et al., 2011]. Common CNVs identified in this study (online suppl. table 1) need further efforts to

identify robust signatures (independent of the 13 CNVs we reported here) through replication studies, as outlined for GWAS study designs.

Putative Functional Relevance of the Identified CNVs

Recent interest in CNVs is also on their *cis*-regulatory potential in controlling the expression of nearby genes and therefore the phenotypes. The loss or gain of altered transcription factor binding sites or enhancer elements within the CNV loci may confer the *cis*-regulation [Schlattl et al., 2011; Haraksingh and Snyder, 2013]. In addition, focus has also been on embedded miRNA, lncRNA, and other small RNA genes transcribed from intergenic and intragenic regions for their obvious roles in post-transcriptional and epigenetic gene regulatory mechanisms [Wu et al., 2012; Meng et al., 2014; Sorensen et al., 2015]. We identified 3 candidate lncRNA genes within or proximal to the CNVs showing association with BC (table 2). Evidence for expression of lncRNA in this region would support the premise that CNVs may in part contribute to the phenotypic variance through the expression of noncoding RNAs regulating protein-coding genes. Independent confirmation of the expression of identified lncRNAs in breast tissues was obtained from the lncRNA expression studies in BC (TANRIC). We compared the normalized counts (output from the database for a queried region) in matched normal and invasive breast tumor tissues ($n = 105$ pairs from TCGA) for a representative lncRNA (RP11-206M11.7; table 2). Expression of this lncRNA was observed in tumor tissues (average of 100 normalized counts) as well as in adjoining normal tissues (average of 52 normalized counts), indicating that the CNVR encompassing this lncRNA is transcriptionally active and is indeed differentially expressed in breast tissues. Further, this lncRNA has been reported as a promising prognostic factor for BC [Meng et al., 2014; Sorensen et al., 2015]. The remaining 2 lncRNAs from chromosome 5 showed low or negligible expression in breast tissues (data not shown). However, we did not identify miRNA genes from the limited number of CNV targets interrogated (listed in table 2).

Discussion

We identified common CNVs from germline DNA as potential susceptibility variants for BC risk. Thirteen CNVs called by Nexus and Partek showed overlap and were associated with BC. These signatures were identified

based on concordances for frequencies, effect sizes, and direction (gain or loss) between the 2 algorithms. This is also the first comprehensive study in sporadic BC to mine CNVs utilizing 2 CNV calling algorithms.

Subsets of CNV loci identified (table 2) harbored genes that are known to play a role in BC: (1) *NBPF9* and an adjacent gene, *PDE4DIP*, are evolutionarily conserved and exhibit CNVRs; these gene loci are mutated in BC and in neuroblastoma [Diskin et al., 2009; O’Bleness et al., 2012; Horvath et al., 2013]; (2) the *AUTS2* gene was shown to be prognostic for BC recurrence and is also an integral member of the PAM50 prognostic gene cluster [Chanrion et al., 2008; Stadler et al., 2012; Nagamani et al., 2013]; (3) *ASTN2* gene rearrangements are prognostic in BC [Glessner et al., 2009; Hampton et al., 2009]; and (4) *SGCD* is known to mediate paclitaxel sensitivity [Conrad et al., 2010b; Eng et al., 2011; Beck et al., 2013; Chet- tier et al., 2014].

CNVs also potentially harbor *cis*-regulatory regions and small RNAs and as such are attractive tools [Vaish- navi et al., 2013] to explain the functional relevance for the BC susceptibility variants identified, and the same was demonstrated in this study. A CNV in chromosome 3q25.1 shown to be associated with BC also harbored lncRNA and showed expression in a tissue-specific man- ner (table 2). Correlations of expression with the copy number status in breast tumor tissues remain to be deter- mined. Evidence of breast tissue-specific expression and putative functional significance of CNV loci associated with disease phenotypes complement the approach of validating CNV calls by 2 independent algorithms and lend confidence in the study findings.

In addition to the observed associations with BC phe- notype, the identified CNV regions also were shown to be associated with other diseases (show pleiotropy) such as autism, schizophrenia, multiple sclerosis, and congenital cardiovascular disease [Vandepoele et al., 2005; Glessner et al., 2009; Kao et al., 2010; Stadler et al., 2012; Lalani et al., 2013; Nagamani et al., 2013; Szatkiewicz et al., 2014; Li et al., 2016].

Interestingly, a higher prevalence of autism correlates with a high incidence of in situ BC [Kao et al., 2010]. Au- tism and incidence of cancers share a common feature, i.e., a high rate of genomic aberrations underlying disease etiology. The above findings are from as of now limited knowledge in recognizing the pleiotropy of gene-disease relationships and underpin the paucity of literature on the shared predisposing mutations, SNPs and CNVs, which play a complex role in conferring disease etiolo- gies.

Table 2. Nexus CNVs are compared for concordance of statistical significance and effect size with Partek signatures

Chr	Nexus start	stop	Cytoband	Copy number	Region length, bp	Cases, n	Controls, n	Frequency		p value	Q	Genes	Odds ratio (95% CI)	Overlap with DGV, %	Gencode annotation for lncRNA (gene)
								cases	controls						
1	145004248	145041078	q21.1	gain	36,830	56	87	15.56	27.19	2.28E-04	7.94E-03	LINC00305, LOC284294, NBPF9, PDE4DIP	0.5 (0.34–0.72)	100	none
2	3780487	3820219	p25.3	gain	39,732	37	8	10.28	2.50	3.76E-05	1.84E-03	none	4.47 (2.05–9.74)	100	none
2	183256977	183284352	q32.1	loss	27,375	2	34	0.56	10.63	6.17E-10	1.42E-07	PDE1A	0.04 (0.01–0.19)	100	none
3	148992514	149021147	q25.1	gain	28,633	50	16	13.89	5.00	8.20E-05	3.43E-03	none	3.1 (1.7–5.49)	100	chr3:149002569-149051440 (RP11-206M11.7)
5	74279721	74302658	q13.3	gain	22,937	72	20	20.00	6.25	1.39E-07	1.51E-05	none	3.75 (2.23–6.31)	100	chr5:74213551-74319753 (CTD-250S016.4)
5	106226427	106233130	q21.3	loss	6,703	9	37	2.50	11.56	2.19E-06	1.83E-04	LOC102467213	0.19 (0.09–0.41)	100	chr5:106150898-106346715 (CTC-254b4.1)
5	136913980	136955707	q31.2	gain	41,727	35	12	9.72	3.75	2.26E-03	5.41E-02	KLHL3	2.76 (1.41–5.42)	100	none
5	155456936	155475101	q33.2	gain	18,165	27	8	7.50	2.50	3.02E-03	6.71E-02	SGCD	3.16 (1.41–7.06)	100	none
9	120100872	120123796	q33.1	gain	22,924	4	32	1.11	10.00	1.18E-07	1.31E-05	ASTN2	0.1 (0.04–0.29)	100	none
12	26578673	26606370	p11.23	loss	27,697	64	20	17.78	6.25	3.96E-06	3.01E-04	AUTS2	3.24 (1.91–5.49)	8	none
12	55725882	55737478	q13.2	loss	11,596	9	25	2.50	7.81	2.26E-03	5.30E-02	none	0.3 (0.14–0.66)	100	none
16	65902385	65911443	q21	gain	9,058	42	2	11.67	0.63	2.11E-10	6.05E-08	none	21 (5.04–87.49)	100	none
21	15070104	15091284	q11.2	gain	21,180	76	32	21.11	10.00	8.87E-05	3.69E-03	none	2.4 (1.6–3.8)	100	none

Shown in the table are the 13 Nexus signatures concordant with Partek GS-called signatures. Concordances shown are for effect size, differential frequencies, copy number gain or loss events, and statistical significance. CNV start and stop positions were queried in the UCSC browser for the presence of noncoding RNAs. Long noncoding RNA (lncRNA) gene coordinates and nomenclature adopted are from the GENCODE project. Chr = Chromosome.

In the GWAS domain, a multi-stage association study design using large cohorts is needed to confer statistical power, and controlling for population stratification is equally important to reduce false positive associations. We controlled for population stratification, applied filters which are stringent for CNV studies, and utilized 2 different CNV calling algorithms to ensure comprehensive detection of CNVs. Some of the reported CNV regions in this study were indeed characterized as disease conferring across a spectrum of phenotypes and therefore provide confidence in our study findings [Butler et al., 2005; Bailey et al., 2008; Diskin et al., 2009; Glessner et al., 2009, 2010; Ehli et al., 2012; Stadler et al., 2012; Lalani et al., 2013; Nagamani et al., 2013; Ukkola-Vuoti et al., 2013; Chettier et al., 2014; Szatkiewicz et al., 2014; Furuya et al., 2015].

Progress in the detection of CNVs as germline determinants of disease risk has been limited and may be ascribed to the suboptimal performances of the segmentation algorithms in ascertaining the breakpoints, genome-scale validation of CNVs by qRT-PCR not being cost-effective; and limitations on the design of primers for validating complex genomic regions. While comparing 2 algorithms to detect concordant signatures is one approach to narrow down the list of targets for future independent validations, CNVs identified by individual algorithms also deserve attention. Individual algorithms have also been successfully utilized in CNV-GWAS. However, independent cohorts profiled on the same platform or validations of selected CNVs by qRT-PCR are needed for replication of discovery stage findings.

The sample size for cases (BRCA positive or non-BRCA familial) in previous BC CNV studies ranged from 68 to 129, and for controls from 40 to 128. The platform technologies also varied (Illumina, Affymetrix Cyto 2.7 M array, Agilent aCGH) as well as CNV calling algorithms (Agilent, Affymetrix, Nexus, Illumina) [Zhang et al., 2011; Krepischi et al., 2012a; Pylkas et al., 2012; Kuusisto et al., 2013; Masson et al., 2014], making it difficult to compare the signatures across all these studies. Rare CNVs identified to date in familial BC showed little overlap between studies [Krepischi et al., 2012b; Kuusisto et al., 2013; Masson et al., 2014], underscoring the need for large-scale replication studies and further development of robust CNV calling algorithms. The identified common CNV associations described in the present study may help to warrant further work to extend the premise that understanding the heritable nature of BC susceptibility in populations may also reside outside of SNP-based association-mapping strategies.

Conclusion

We identified CNVs associated with BC susceptibility; subsets of these CNVs harbored genes known to play a role in BC etiology or prognosis. A comprehensive search for CNVs in BC predisposition or for any phenotype association studies is an iterative exercise as refinements in calling algorithms offering finer resolution of breakpoints for CNV boundaries will likely unravel additional variants in the future. Using 2 or more algorithms for segmentation is a humble start to limit the number of false positive associations. As such, stage 1 of the current CNV-GWAS offers putative candidate loci for replication in large and independent cohorts.

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Statement of Ethics

Written informed consent was obtained from all the study participants, and The Alberta Cancer Research Ethics Committee (ACREC) approved the study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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