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Recurrent Copy Number Alterations in *BRCA1*-Mutated Ovarian Tumors Alter Biological Pathways

Karin Leunen,[1*†] Olivier Gevaert,[2†] Anneleen Daemen,[2] Vanessa Vanspauwen,[3] Geneviève Michils,[3] Bart De Moor,[2] Philippe Moerman,[4] Ignace Vergote,[1] and Eric Legius[3]

[1]*Division of Gynecological Oncology, Department of Obstetrics and Gynecology, University Hospitals Leuven, Katholieke Universiteit Leuven, Belgium;* [2]*Bioinformatics, Department of Electrical Engineering (ESAT-SCD-SISTA), Katholieke Universiteit Leuven, Belgium;* [3]*Department of Human Genetics, University Hospitals Leuven, Katholieke Universiteit Leuven, Belgium;* [4]*Department of Pathology, University Hospitals Leuven, Katholieke Universiteit Leuven, Belgium*

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ABSTRACT: Array CGH was used to identify recurrent copy number alterations (RCNA) characteristic of either BRCA1-related or sporadic ovarian cancer. After preprocessing, both groups of patients were modeled using a recurrent Hidden Markov Model to detect RCNA. RCNA with a probability higher than 80% were called. After removing RCNA present in both groups, the genes present in the remaining RCNA were investigated for enrichment of pathways from external databases. More RCNA were observed in the BRCA1 group, and they display more losses than gains compared to the sporadic group. When focusing on the type of RCNA, no significant difference in length was seen for the gains, but there was a statistically significant difference for the losses. In the sporadic group, a great proportion of the altered regions contain genes known to have a function in cell adhesion and complement activation, whereas the BRCA1 samples are characterized by alterations in the HOX genes, metalloproteinases, tumor suppressor genes, and the estrogen-signaling pathways. We conclude that BRCA1 ovarian tumors present a different type, number, and length of RCNA; a huge amount of the genome is lost, resulting in important genomic instability. Moreover, important biological pathways are altered differentially when compared to the sporadic group.
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KEY WORDS: array CGH; BRCA1; ovarian cancer; hidden Markov model; recurrent copy number alterations

Introduction

Array comparative genomic hybridization (aCGH) has gained more interest in the last years, and is now widely used in oncologic research [Davies et al., 2005]. The main advantage of aCGH compared to traditional CGH is that this technology measures variations in DNA copy number at a genome wide scale and at a much higher resolution.

The aim of this study was to look for differences in gains and losses in BRCA-related ovarian cancers compared with sporadic ones. More specifically, we set out to identify recurrent copy number alterations (RCNA) characteristic of either *BRCA1*-mutated or sporadic ovarian cancer. These RCNA are defined as regions of the genome that are altered in all samples within a group (e.g., BRCA1 or sporadic). Identifying RCNA characteristic of either tumor type can elucidate possible distinct pathways between these patients and allows us to learn more about their oncogenesis. Many previous studies investigated copy number alterations (CNA) in sporadic and BRCA-related ovarian tumors using traditional low-resolution CGH [Patael-Karasik et al., 2000; Tapper et al., 1998; Zweemer et al., 2001], but comparison between both groups using aCGH to identify RCNA has not yet been reported. Identifying RCNA characteristic of each tumor type can elucidate possible distinct pathways between these patients and allows us to learn more about their oncogenesis. Recently, Walsh et al. [2008] did a similar genome wide analysis in which they studied a similar number of patients. However, they used a single nucleotide polymorphism (SNP) array to investigate loss of heterozygosity (LOH) and uniparental disomy (UPD), and did not identify RCNA specific to both groups of tumors. Although our study design is different from the one of Walsh et al. [2008], we confirm an enhanced instability due to percentage of genome altered in the BRCA group compared to the sporadic ovarian cancers.

Here we report the results of our genome-wide characterization of RCNA in BRCA1-mutated versus sporadic ovarian tumors using aCGH. We focus on the type of RCNA, their number and length, as a basis in our search to demonstrate that distinct biological pathways might be active in sporadic and hereditary ovarian cancer.

Materials and Methods

Sample Description

Data from 13 patients treated for ovarian cancer at the University Hospital of Leuven, Belgium (five BRCA1-related and eight sporadic ovarian cancers) were collected for participation in this study. To make the sporadic ovarian cancer patients as similar as possible to the BRCA mutated ovarian cancer patients only patients with similar clinical and pathologic characteristics were studied: they were all stage III–IV, serous papillary poorly

Additional Supporting Information may be found in the online version of this article.

†The first two authors contributed equally to this article.

*Correspondence to: Karin Leunen, Department of Gynecology and Obstetrics, Division of Gynecologic Oncology, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. E-mail: karin.leunen@uz.kuleuven.ac.be

differentiated ovarian cancers. All tumor samples were collected at the time of primary surgery and immediately rinsed and frozen in liquid nitrogen. All patients signed an informed consent to perform this analysis, which was approved by the ethical committee of the University Hospitals Leuven. In aCGH, the number of malignant cells in each sample determines the sensitivity of the technique. Therefore, to reduce the sensitivity to noise and to limit stromal contamination, which also decreases significantly the sensitivity for detecting copy changes, histological confirmation of a high percentage of tumor load (at least 70%) was confirmed by a pathologist. DNA was extracted from the fresh frozen samples. Each tumor was hybridized twice (dye-swap) against a common reference pool. The reference cell population was extracted from peripheral blood samples of each matched participating patient.

ArrayCGH and Preprocessing

After extraction of DNA, tumor DNA was labeled by a random priming method (Bioprime® DNA labeling system; Invitrogen, Cergy-Pontois, France) with cyanine-5. Reference DNA was similarly labeled with cyanine-3. After purification, coprecipitation with 50 µl Cot-1 DNA (Invitrogen), resuspension in hybridization buffer (20% formamide), denaturation at 75°C for 10 min and prehybridization at 37°C for 60 min were subsequently performed. Probes were cohybridized on aCGH. The aCGH slide was previously preblocked with a buffer. We used a 1-Mb BAC array CGH platform, version CGH-SANGER 3 K 7 developed by the Flanders Institute for Biotechnology (VIB), Facility, Leuven, Belgium, and the Center of Human Genetics, Leuven, Belgium. After washing, arrays were scanned using a 4000B scanner (Axon, Union City, CA, USA). Genepix Pro 6.0 software (Axon) was used for image analysis and data were further analyzed by Microsoft Excel. First, Cy5 and Cy3 fluorescence intensities were background corrected. Then, the ratio of the Cy5 to Cy3 was calculated and normalized by the median Cy5–Cy3 ratio. Finally, these ratios were log transformed and the average of the replicate (dye-swap) experiments was used as input for subsequent modeling.

Recurrent Hidden Markov Model

To identify differential regions between the BRCA1 and sporadic patients, these samples were analyzed using Hidden Markov Modeling (HMM) [Lai et al., 2005; Shah et al., 2006]. In brief, using this methodology each sample is modeled by a HMM with three hidden states corresponding to copy number loss (CNL), neutral and copy number gain (CNG). An HMM naturally models CNA because it allows to take into account the genomic neighborhood. Instead of modeling each sample separately with an HMM, we grouped samples according to their BRCA1 status with a recurrent HMM (RHMM) [Shah et al., 2007]. This allows identification of RCNA found at the same location in multiple samples using a statistical model. The recurrent HMM delivers the probability of RCNA across all samples belonging to the same group. A recurrent CNL (RCNL) or recurrent CNG (RCNG) was called when its probability of occurring was more than 80%.

Statistical Analysis of RCNA

Next, we compared the RCNA of both groups according to their number, type (i.e., RCNG or RCNL) and length. We used the Wilcoxon rank sum test to assess statistical significance. All tests were two sided and the significance threshold was 0.05.

Pathway Enrichment Analysis

Finally, the RCNA were subjected to pathway enrichment analysis. First, because we aimed to investigate differential pathways between both groups of tumors, the RCNA overlapping between the BRCA1 and the sporadic samples were removed. Then, the HUGO identifiers from all genes within the remaining RCNA were extracted using the Ensembl database [Hubbard et al., 2009]. The genes resulting from this operation were called a signature. Next, we extracted all curated pathways from the MSigDB database v2.1 [Subramanian et al., 2005]. These curated pathways come from well-known pathway databases such as KEGG or Biocarta. The enrichment analysis was carried out by calculating the number of genes overlapping between each curated pathway and the genes contained in the RCNA (i.e., the signature). Next, 5,000 random signatures of the same size were constructed and the overlap between the curated pathway and the randomly constructed signatures was calculated. A *P*-value was subsequently determined by counting the number of more extreme observations in the random set of signatures compared to the number of genes overlapping with the real signature [North et al., 2002]. Due to the large number of statistical tests that is performed, multiple testing correction was performed by controlling the false discovery rate (FDR) [Benjamini and Hochberg, 1995].

Results

Identification of RCNA

After preprocessing, we applied RHMM-modeling on both the BRCA1 and sporadic samples separately. This resulted in two sets of RCNA characterizing the BRCA1 and sporadic group. Figure 1 shows the probability of a RCNA on chromosome 1 for the BRCA1 and sporadic group on chromosome 1 (see Supp. Fig. S1–S23 for the probabilities of RCNA on all other chromosomes). The probability of RCNG and RCNL are indicated in green and red, respectively. RCNG and RCNL with a probability of 80% or more are shown on top of the raw probabilities. These RCNA are "called," and will be used for further analysis.

Statistical Analysis of RCNA

Based on these called RCNA, we investigated whether there is a difference in the number, the length, and the type of RCNA between both groups. The number of breakpoints was similar between the two groups. There were 178 breakpoints in the BRCA1 tumors compared to 164 breakpoints in the sporadic patients. Figure 2A shows the number of breakpoints for each chromosome and according to each patient group.

When focusing on the type of RCNA, the sporadic group had the most RCNG, whereas the BRCA1 group had the most RCNL. More specifically, the BRCA1 group is characterized by 98 RCNA corresponding to 34 RCNG and 64 RCNL, compared to 86 RCNA in the sporadic group corresponding to 40 RCNG and 46 RCNL.

Next, we focused on the length of the RCNA. We did not observe an overall difference in length between the two groups. When focusing on the type of RCNA, however, there was a statistically significant difference between the lengths of the RCNL. The RCNL in the BRCA1 group were typically longer with a median length of 5.2 Mb in the BRCA1 group versus 0.2 Mb in the sporadic group (*P*-value $<$ 1.8e-8; Fig. 2B). The RCNG were also longer in the BRCA1 group; however, this difference was not

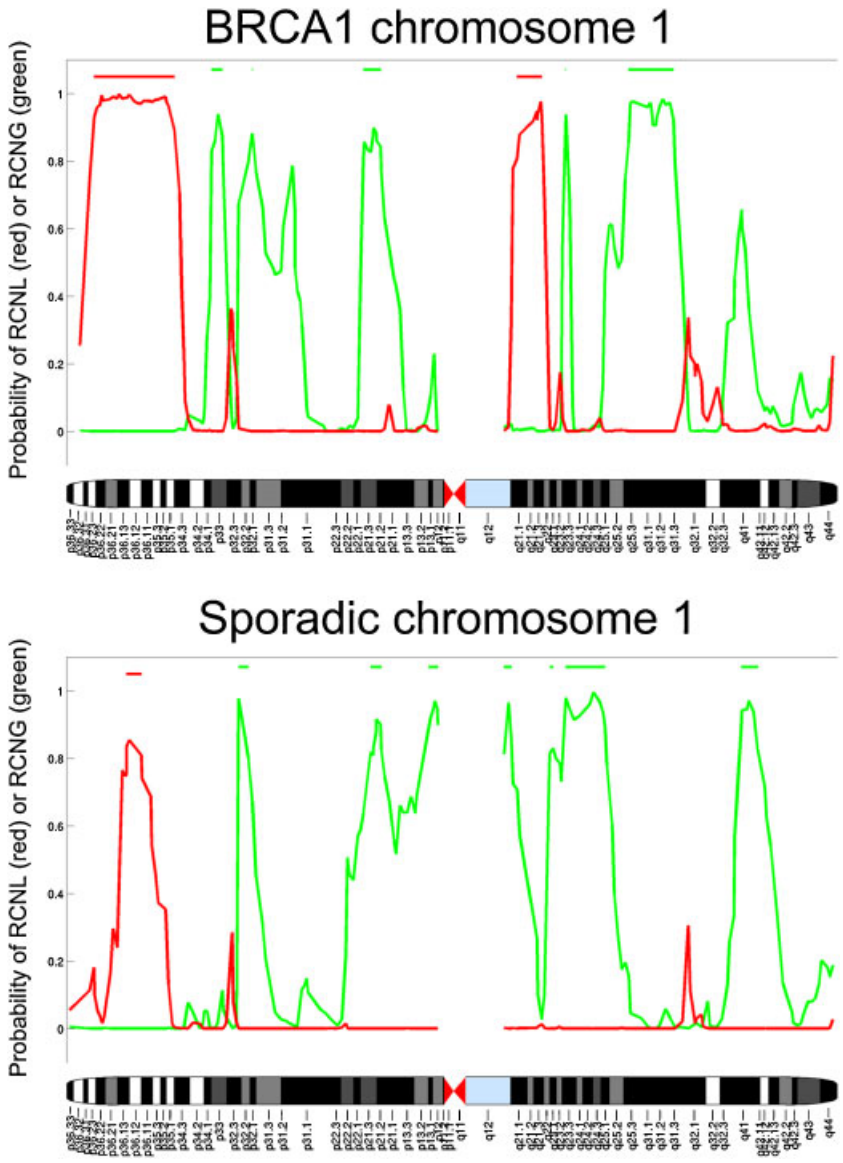

Figure 1. Probabilities for recurrent copy number loss (RCNL) in red and recurrent copy number gains (RCNG) in green after recurrent Hidden Markov modeling (RHMM). **Top panel:** RCNL and RCNG on chromosome 1 for the BRCA1 group. **Bottom panel:** RCNL and RCNG on chromosome 1 for the sporadic group. RCNG and RCNL with a probability of 80% or more are shown on top of the raw probabilities and were further analyzed.

statistically significant (median length of 5.3 Mb and 2.5 Mb for the BRCA1 and sporadic group, respectively; Fig. 2C).

Figure 3 further shows the number and median length of the RCNG and RCNL per chromosome. When focusing on the median length of the RCNL, Figure 3D confirms the above-mentioned results because the RCNL are longer in most chromosomes of BRCA1-mutated tumors, indicating that this difference is not due to a large RCNL on a single chromosome.

In total 730 Mb or ~22% of the haploid human genome is affected by RCNA in BRCA1 tumors (257 Mb gained and 473 Mb lost) compared to 475 Mb or ~15% in sporadic tumors (384 Mb gained and 91 Mb lost). When considering all differential RCNA (i.e., after removing overlapping RCNA between both groups of samples), up to 6,581 genes were affected. When focusing only on the RCNL, 4,775 genes were affected in the BRCA1 group, in contrast to only 327 genes in the sporadic group. Similarly, 491 genes and 988 genes were found in the RCNG of the BRCA1 and sporadic group, respectively.

It is important to state that different thresholds for recurrent aberrations in the range of 50% to 90% for calling RCNA all resulted in a significantly larger length of RCNL in BRCA1 tumors compared to sporadic tumors. This indicates that these results do not depend on the threshold for calling RCNA.

To summarize the results of the RCNA, a karyogram for both the sporadic as well as the BRCA-related ovarian cancer group, is displayed in Figure 4. Visual comparison shows obvious differences for chromosomes 5q, 7, and 15–19 where deletions are more frequent than in the sporadic group. Looking at the gains, those in 2q and 10q are striking in the BRCA-related group, whereas in the sporadic group gains are seen in 3q, 5p, 8q, and 20q.

Pathway Enrichment Analysis

Because we hypothesized that interesting biological processes might be disrupted by these RCNA, we further investigated these signatures. Manual annotation of these genes is not practical.

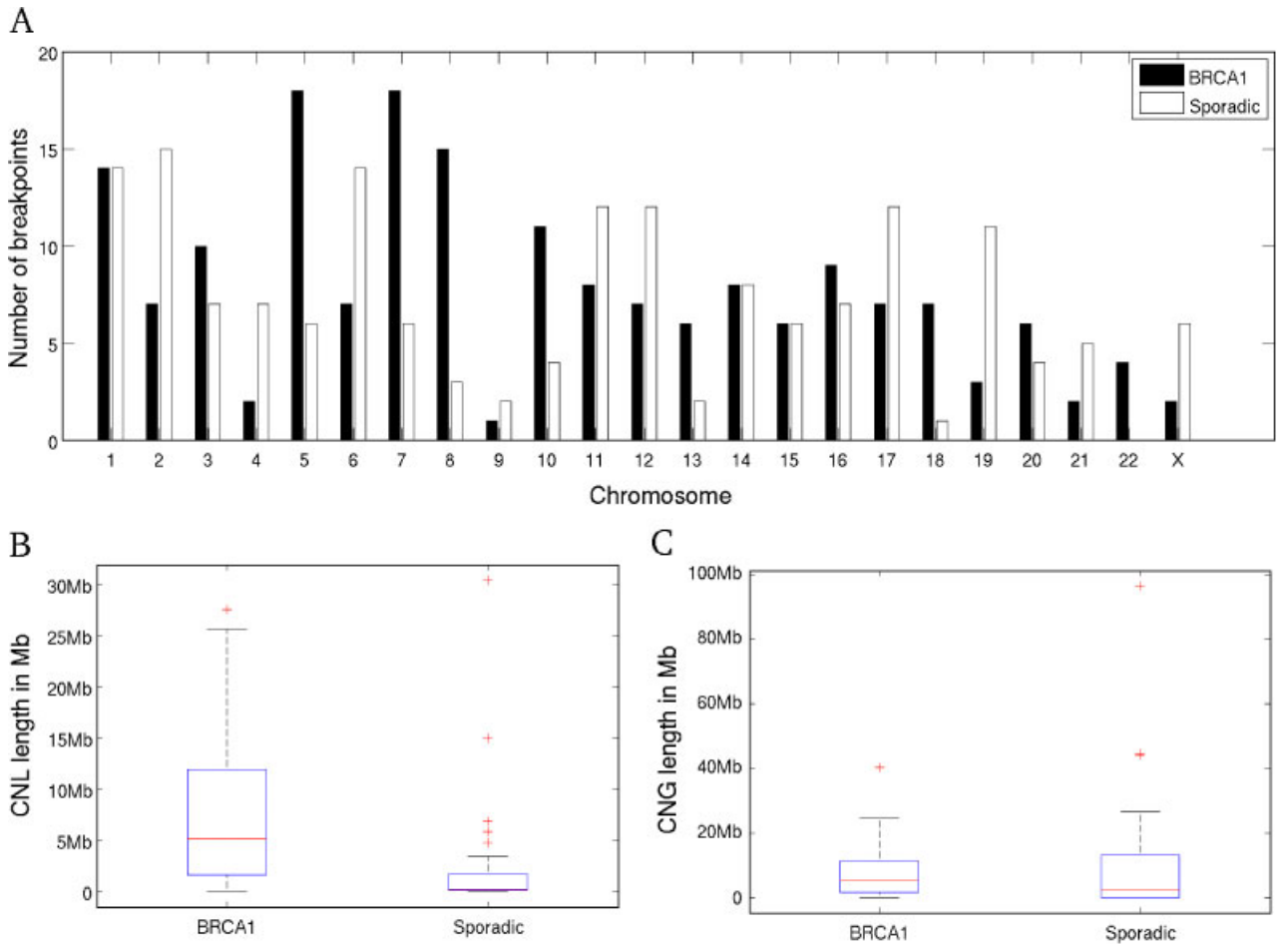

Figure 2. Visualization of the number of breakpoints per chromosome and the length of all recurrent copy number losses (RCNL) and gains (RCNG) separately for the BRCA1 and sporadic patient groups. **A:** Number of breakpoints per chromosome; **B:** boxplot of length of RCNL; **C:** boxplot of length of RCNG.

Therefore, we resorted to pathway enrichment analysis by checking the overrepresentation of pathways from publicly available databases with our signatures. All pathways were downloaded from MSigDB and were constructed based on well-known pathway databases such as KEGG or Biocarta [Subramanian et al., 2005]. We chose the HUGO gene names as the common identifier because the MSigDB database uses this identifier to uniquely characterize genes.

Table 1 lists the most important pathways that are enriched in the RCNA specific to the BRCA1 group (see Supp. Table S1 for a full list of the significant pathways). As shown in this table, the HOX genes are altered in the BRCA1 group. More specifically, a part of this pathway is gained and another part is lost. Also, a collection of tumor suppressor genes is lost, whereas a set of matrix metalloproteinases are gained. Next, genes related to estrogen signaling are lost. Subsequently, the pathway responsible for methylation of CARM1 through estrogen signaling is significantly lost in BRCA1 patients.

Finally, Table 2 shows the most important pathways that are specifically enriched in the sporadic group (see Supp. Table S2 for a full list of the significant pathways). For the sporadic patients no pathways were found that were lost. However, a cancer-related pathway involved in cell adhesion and metalloproteinases is altered. Again, most of these genes appear to be related to cell adhesion and are not metalloproteinases, corresponding with the enrichment of the GO category cell adhesion. Finally, a gene set containing experimentally identified targets of the oncogene MYC are also gained in sporadic patients.

Census of Cancer Genes

Finally, we refer to Supp. Table S3 for RCNA status of the list of genes known as "census of cancer genes," already described in the literature [Futreal et al., 2004]. Listing the genes retained in our patient cohort gives the opportunity to compare them with data collected by other investigators and maybe in the future, to better acknowledge the relative importance of each gene in different tumors.

Discussion

In the present study we compared sporadic and BRCA1-related ovarian cancer using aCGH. There is growing evidence that both of these ovarian cancers have a different oncogenetic origin, and that different tumor biology reflects distinct pathways in both groups. If this hypothesis is correct, this means that different downstream genes in these pathways are involved and that their identification can lead to a distinctive clinical management of these two types of ovarian cancers.

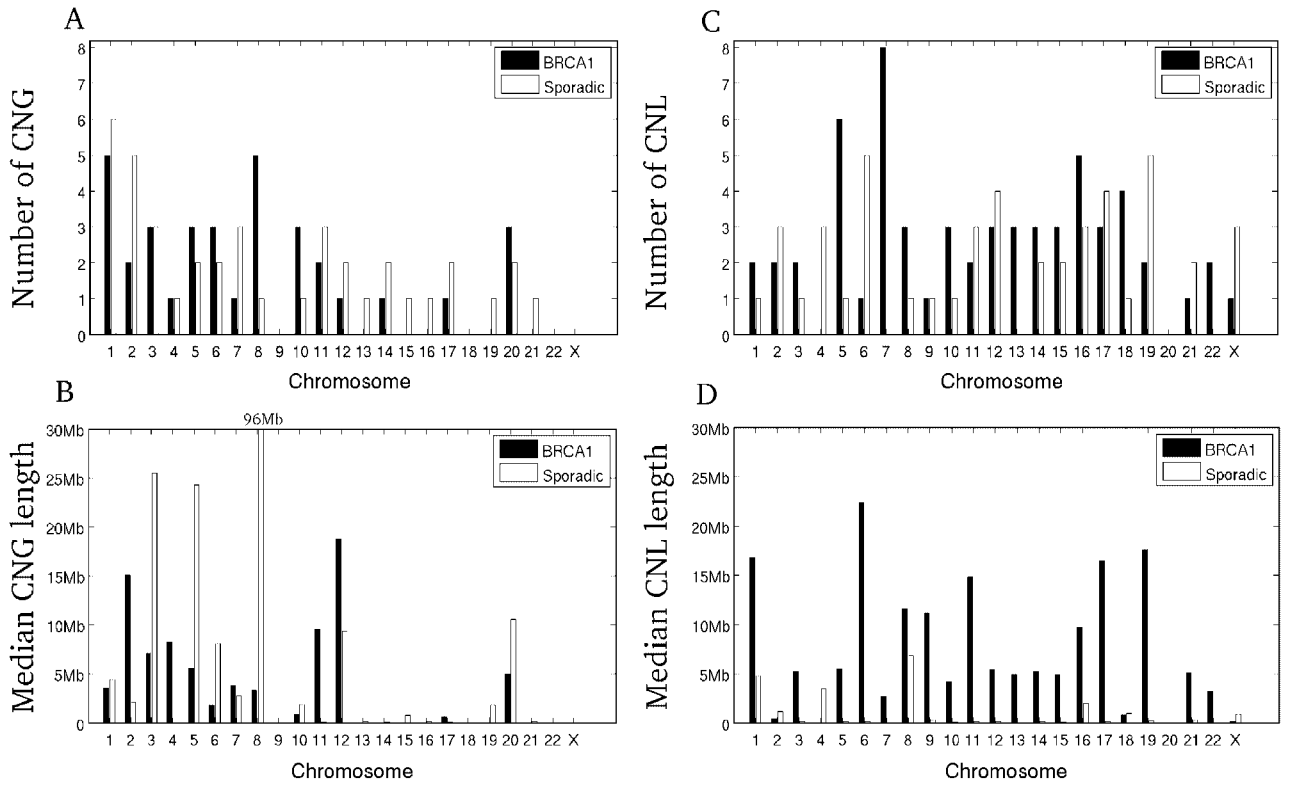

Figure 3. Visualization of the number and length of recurrent copy number gains (RCNG) and losses (RCNL) per chromosome separately for the BRCA1 and sporadic patient groups. **A:** Number of RCNG; **B:** median length of RCNG; **C:** number of RCNL; **D:** median length of RCNL. The 8q gain of 96 Mb in sporadic patients is not completely visualized such that y-axis length is similar in panels B and D.

The aCGH technique is gaining interest, and is currently used in many studies in most cancer types. The use of aCHG to compare sporadic with BRCA1-related ovarian cancers, however, has been until now, poorly explored. Moreover, the statistical analysis based on RHMM used here as well as its use in a search to identify differential pathways in sporadic versus BRCA1-related ovarian cancers is innovating and has not been used before in this context. The main advantage is that the identification of CNA is performed recurrently instead of in a postanalysis.

Previously, a few small studies using metaphase CGH to compare sporadic with hereditary ovarian cancer have been done. Different pathways in the oncogenesis of both groups have been suggested [Israeli et al., 2003; Patael-Karasik et al., 2000; Tapper et al., 1998; Zweemer et al., 2001] but using aCGH we were able to profile, validate, and refine RCNA at a much higher resolution. Moreover, once RCNA were identified, the collected data were used to unravel distinct pathways, reflecting different carcinogenetic processes.

Our results indicate that the RCNA are distinct in sporadic versus hereditary ovarian cancers. Losses are more frequent in the BRCA1 group. This can be expected due to the role of BRCA1 in homologous repair of double-stranded DNA breaks, but was never demonstrated before using high-resolution technology. This suggests that most RCNA encompass tumor suppressor genes rather than oncogenes. Additionally, the recurrent losses are longer in the BRCA1 group (5.2 Mb vs. 0.2 Mb), suggesting that large parts of the genome are absent in the hereditary group and a huge amount of genetic material is lost (in total, 473 Mb). This loss of genetic material in the BRCA1 patients contributes to the important genetic instability of these hereditary tumors. However, it should be underscored that the evaluation of the length of the RCNA is in a sense virtual, because we focused on recurrent changes; therefore, the study of the length of these RCNA should be interpreted in that context. Additionally, the lengths of RCNA can be smaller than the resolution of the array due to RCNA of a single clone (scored as 0.1 Mb).

The loss of genetic material confirms recent findings of the group of Walsh et al. [2008]. In their series, entirety of chromosome 13 and 17 are affected almost exclusively by UPD or deletion. However, when compared to our data, their observed amplifications present at a greater frequency in the BRCA-associated tumors and at a relatively evenly distributed frequency across the genome, whereas our results point out relatively more RCNL than RCNG in the BRCA group.

Looking at the localization of the RCNA on the genome, previous reports using metaphase CGH showed that gains in 8q and 3q were often present [Zweemer et al., 2001] but not qualifying as specific for the sporadic group as shown in our results (see Supp. Figs. S1–S23). Both chromosome regions harbor genes known as *c-MYC* (MIM# 190080), tyrosine kinase *LYN* (MIM# 165120) and threonine kinase *MOS* (MIM# 190060) on 8q as well as *PIK3CA* (MIM# 171834) and tyrosine kinase *RYK* (MIM# 600524) on 3q. *Myc* is overexpressed in more than 30% of ovarian cancers. *PIK3CA* (located at the 3q26 amplicon) has an important function in signal transduction, and has been shown to be involved in ovarian carcinogenesis [Shayesteh et al., 1999].

On a higher 550 K SNP array resolution, Haverty et al. [2009] only studied sporadic ovarian cancer and also demonstrated the most prevalent gains on 8q and 3q, whereas for deletions, the peaks on p-arms of chromosome X and 8 were the dominant features. Also, in breast cancer cell lines, multiple alterations in chromosome 8 have been described, including complete

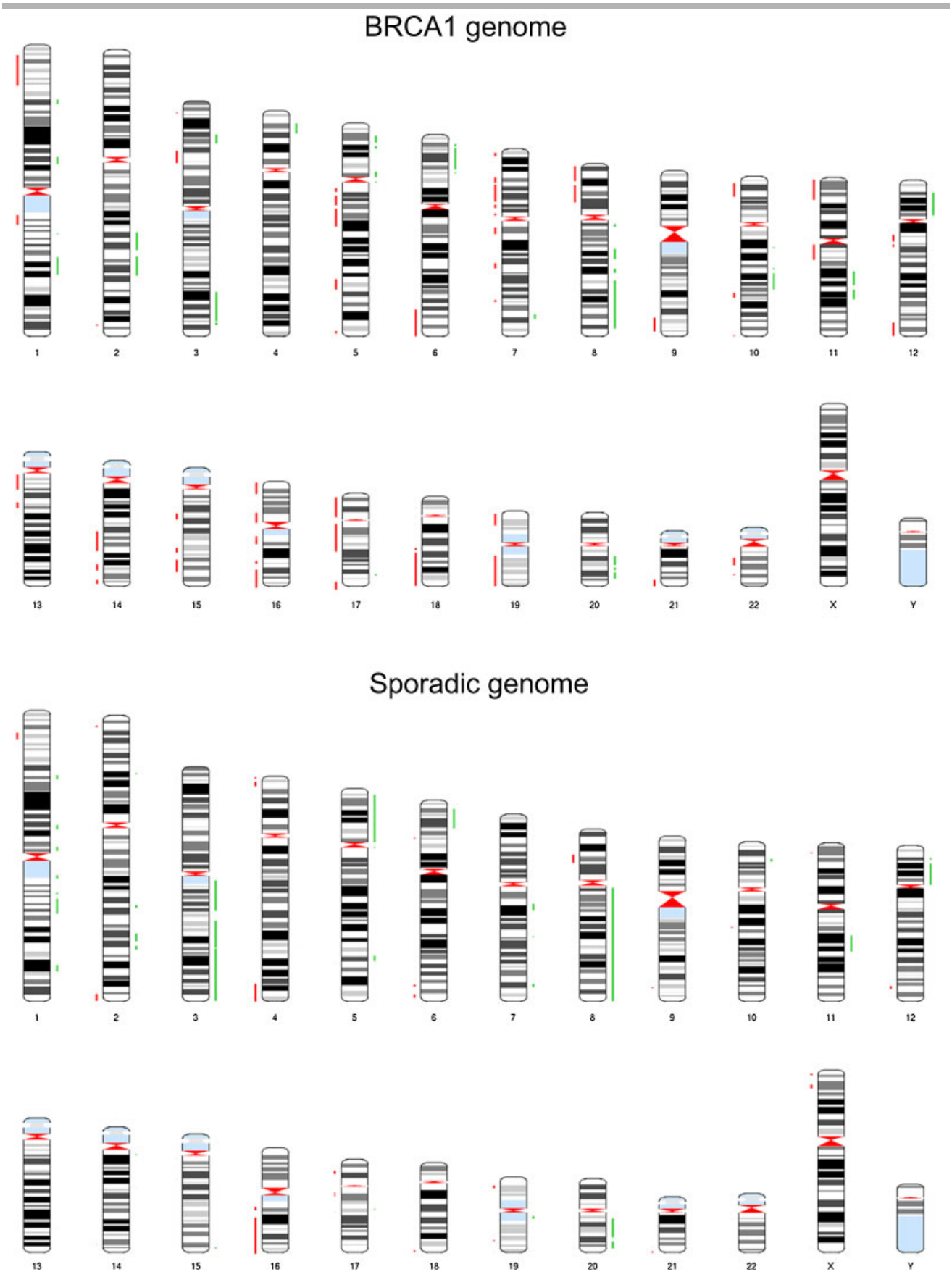

Figure 4. Karyogram displaying the recurrent copy number gains (RCNG) in red and losses (RCNL) in green for the BRCA1 and sporadic patient groups.

Table 1. The Most Important Pathways Enriched in the Genes Overlapping with the RCNA in the BRCA1 Tumors

Signature	Gene set name from MSigDB	*P*-value	Q-value	Overlapping genes
BRCA1-either	HOX GENES	0.00020	0.02074	HOXA6 CBX8 LHX2 HOXD10 HHEX HOXB5 HOXD11 HOXB13 HOXA5 EZH1 HOXD9 HOXA2 HOXD13 HOXA4 PHC2 HOXA11 HOXA1 HOXD1 CBX4 HOXD12 HOXB3 HOXA3 DLX4 HOXA10 HOXB2 HOXD4 HOXB7 HOXA7 HOXD3 HOXB1 HOXB9 HOXA9 HOXB6
BRCA1-either	BREAST CANCER ESTROGEN SIGNALING	0.00080	0.04609	SPRR1B ATF2 CLDN7 PTGS2 TP53 GATA3 ERBB2 CCND1 SCGB1D2 THBS2 CDKN1B C3 KLK5 FOSL1 KRT18 DLC1 KRT19 CTSB IL6ST RPL27 FLRT1 NGFR SERPINE1 IL2RA SCGB2A2 BCL2 HMGB1 SCGB2A1 TNFAIP2 AZGP1 ESR1 EGFR ESR2 RPL13A S100A2 SERPINB5 PGR THBS4 BAD COL6A1 ACTB
BRCA1-either	TUMOR SUPPRESSOR	0.00100	0.04609	BRCA2 CDKN2D BRCA1 LCMT2 EP300 CDKN1B TSC2 CDKN1C CFL1 TGFBR2 TP53 RB1 NF2 CREBBP ACTB
BRCA1-RCNG	HOX GENES	0.00020	0.08684	HOXD10 HHEX HOXD11 HOXD9 HOXD13 HOXD1 HOXD12 HOXD4 HOXD3
BRCA1-RCNG	MATRIX METALLOPROTEINASES	0.00020	0.08684	MMP3 MMP10 MMP13 MMP27 MMP1 MMP20 MMP7 MMP8 MMP12
BRCA1-RCNL	CARM ERPATHWAY	0.00080	0.05894	ESR1 CARM1 CCND1 HDAC1 BRCA1 HDAC3 EP300 SRA1 GTF2F1 POLR2A HDAC5 TBP NCOR2 CREBBP
BRCA1-RCNL	BREAST CANCER ESTROGEN SIGNALING	0.00180	0.09824	SPRR1B CLDN7 TP53 GATA3 ERBB2 CCND1 SCGB1D2 THBS2 C3 KLK5 FOSL1 KRT18 DLC1 KRT19 CTSB IL6ST RPL27 FLRT1 NGFR SERPINE1 IL2RA SCGB2A2 BCL2 HMGB1 SCGB2A1 TNFAIP2 AZGP1 ESR1 EGFR ESR2 RPL13A S100A2 SERPINB5 THBS4 BAD COL6A1 ACTB
BRCA1-RCNL	TUMOR SUPPRESSOR	0.00200	0.09824	BRCA2 CDKN2D BRCA1 LCMT2 EP300 TSC2 CDKN1C CFL1 TP53 RB1 NF2 CREBBP ACTB
BRCA1-RCNL	HOX GENES	0.00223	0.09852	HOXA6 CBX8 LHX2 HOXB5 HOXB13 HOXA5 EZH1 HOXA2 HOXA4 PHC2 HOXA11 HOXA1 CBX4 HOXB3 HOXA3 DLX4 HOXA10 HOXB2 HOXB7 HOXA7 HOXB1 HOXB9 HOXA9 HOXB6

A complete set of enriched gene sets is available as Supporting Information.
RCNA = recurrent copy number alteration; RCNG = recurrent copy number gain; RCNL = recurrent copy number loss.

Table 2. The Most Important Pathways Enriched in the Genes Overlapping with the RCNA in the Sporadic Tumors

Signature	Gene set name from MSigDB	*P*-value	Q-value	Overlapping genes
Sporadic-either	BRENTANI CELL ADHESION	0.00020	0.01359	ALCAM SELP BYSL GPA33 SELE CDH3 FAT PTK2 CDH6 CDH17 CDH18 CDH12 CDH5 CDH11 CD58 VCAM1 SELL CD47 CDH1
Sporadic-either	CLASSICPATHWAY	0.00020	0.01359	C8A C4B C9 C7 C4A C6
Sporadic-either	COMPLEMENT ACTIVATION CLASSICAL	0.00020	0.01359	C8A C8B C9 C4A C4B C7 C6
Sporadic-either	COMPPATHWAY	0.00020	0.01359	C8A C4B C9 C7 C4A C6
Sporadic-either	ALTERNATIVEPATHWAY	0.00040	0.02378	C7 C8A C9 C6
Sporadic-either	CELL ADHESION	0.00100	0.03964	CNTNAP2 GP5 CD96 ALCAM BYSL CDH16 FAT CD2 CDH17 ITGA8 CDH11 SEMA5A SDC2 CLDN1 CD36 DDR2 MAEA CDH8 NEDD9 CDH3 CDH6 BAI1 CD58 CHST4 SELL
Sporadic-RCNG	SCHUMACHER MYC UP	0.00160	0.05074	UCK2 ACSL1 BOP1 RRS1 DHODH FABP5 TFRC PRPS2 ATP1B3 HSPE1 MRPL3
Sporadic-RCNG	ALTERNATIVEPATHWAY	0.00020	0.01869	C7 C8A C9 C6
Sporadic-RCNG	COMPLEMENT ACTIVATION CLASSICAL	0.00080	0.06409	C8A C8B C9 C7 C6

A complete set of enriched gene sets is available as Supporting Information.
RCNA = recurrent copy number alteration; RCNG = recurrent copy number gain.

or partial deletion of 8p or 8q, duplication of 8q, as well as complex rearrangements, incorporating some potential new tumor suppressor genes [Venter et al., 2005]. This provides evidence that breast and ovarian cancer potentially share the same pathways.

Additionally, a differential higher proportion of gains at 11q22, 13q22, and 17q23–25 were described in the hereditary group [Zweemer et al., 2001]. The gains at 11q22 and 17q25 were confirmed by our data, whereas 13q22 was only gained in a subset of tumors. Zweemer et al. [2001] also found deletions at 15q11–15, 15q24–25, 8q21–ter, 22q13 and 12q24, specific to the hereditary ovarian cancer patients. In light of the higher resolution of our data, these deletions can be refined. Only 15q15.2–3, 15q25, 8q22–ter, 22q13.2, and 12q24.32–33 were specifically deleted in our group of BRCA1 patients (see Supp. Figs. S1–S23). Another study by Tapper et al. [1998] described amplification of 2q24–32 as the only statistically significant difference between BRCA and sporadic ovarian cancer. Our data suggest that only regions 2q24.3 and 2q31.2–3 are regions of common overlap that were recurrently gained in the hereditary group.

Next, Gorringe et al. [2007] identified more than 380 small regions of gain or loss in sporadic ovarian cancer using a 500 K microarray. These small CNA could partially be confirmed in our series, using 1 Mb BAC arrays. For example when focusing on the cancer census genes, WRN (MIM# 277700), NUP214 (MIM# 114350), and GPHN (MIM# 603930), are lost in BRCA1 in our analysis, whereas they are reported as gained in the sporadic group of Gorringe et al. [2007]. Next, RB1 (MIM# 180200) and CBFB (MIM# 121360), reported as losses by Gorringe et al. [2007], are only lost in the BRCA1 and sporadic groups, respectively. In addition, KRAS (MIM# 190070) is gained and MAP2K4 (MIM# 601335) is lost in both groups corresponding to the findings of

Gorringe et al. [2007], whereas GAS7 (MIM# 603127), reported as gained by Gorringe et al. [2007], is lost in both groups in our analysis. However, Gorringe et al. [2007] did not report how frequent each of these aberrations was, which might explain the discrepancies. See Supp. Table S3 for the status of all cancer census genes in our analysis.

In our series, recurrent losses are more clearly present than recurrent gains in contradiction to the CGH findings of Zweemer et al. Moreover, the lengths of RCNA reveal that BRCA related ovarian cancers present significantly more breakpoints in chromosome 5, 7, 8, 10, and 18, whereas chromosome 2, 4, 6, and X harbor a larger number of breakpoints in the sporadic group. Restriction to the RCNL highlights chromosomes 5, 7, and 13 for the BRCA1 group, whereas most RCNL in the sporadic group are located on chromosomes 4, 6, 19, and X (see Figs. 3 and 4).

In 2002 Jazaeri et al. [2002] evaluated 61 tumor samples (18 BRCA1, 16 BRCA2, 27 sporadic) by cDNA microarrays and suggested that BRCA1 and BRCA2 related pathways are also involved in sporadic ovarian cancers. Six of the 53 differentially expressed genes in their study, were mapped on Xp11.23. This region seems to harbor candidate genes interacting with BRCA1 or being regulated by BRCA1, and in this way is involved in ovarian carcinogenesis. Our data could not confirm these data on Xp11 (see Supp. Figs. S1–S23) possibly due to a lack of correspondence between gene expression data and copy number data. However, distinct RCNL were seen at Xp22 in the sporadic group when compared to the BRCA1-related tumors.

In a review of more than 70 studies [Liu and Ganesan, 2002] described 6q, 11q, 13q, 17, 18q, and 22q to be the most common regions of LOH in sporadic ovarian cancers. But due to the presence of copy number neutral LOH, many of these studies found a high frequency of LOH that differed from the reported frequency of copy number losses. In this way, our data lacks information because no difference can been made between true LOH and copy number neutral LOH.

After identification of these RCNA, further analysis by signature construction and pathway enrichment analysis was performed. The power of pathway enrichment lies in the ability to assay many thousands of genes simultaneously and evaluate the multivariate patterns of change across subsets of genes that characterize a physiological or clinical state. In this way, complex patterns can be identified, being typical for a group and reflecting its specific tumor biology [Huang et al., 2003]. If we accept that tumor biology is a result of multiple genes enrolled in pathways, it can be that, when one link is missing in these pathways and not necessarily only the known oncogene is absent, this still leads to alteration or oncogenesis. Knowledge of these pathways may therefore represent targets in prevention or therapy of different cancers such as ovarian cancer.

Tables 1 and 2 are summarizing the important pathways identified in our study, obtained after pathway enrichment on the basis of the RCNA.

In the group of sporadic ovarian cancer patients, a large proportion of the altered regions contained pathways known to have a function in cell adhesion and complement activation, as do *PTK2* (MIM# 600758), *FAT5* (MIM# 600996), *VCAM1* (MIM# 192225), *CDH1* (MIM# 192090), *CDH6* (MIM# 603007), *SDC2* (MIM# 142460), *DDR2* (MIM# 191311), and *NEDD9* (MIM# 602265). The genes *SDC2*, *DDR2*, and *NEDD9*, together with *CDH6* and *FAT*, are similarly correlated to cell adhesion, and in this way play a role in controlling cell growth, migration, and progression of cancer. *PTK2* is a protein kinase implicated in signaling pathways involved in cell motility, proliferation, and apoptosis, and plays a potential role in oncogenic transformation. Together with *MYC*, it is a necessary component for the AKT pathway, accepted actually as a key pathway in the carcinogenesis of ovarian cancer [Crijns et al., 2006; Nowee et al., 2007; Orsulic et al., 2002; Xing and Orsulic, 2006]. MYC, whose role is well known in different cancers and especially in the etiology of a variety of hematopoietic tumors, acts as a master gene for cell growth control and increases transcription of a large variety of genes. It is correlated with *p53* (MIM# 191170) and *Bcl2* (MIM# 151430)-expression [Diebold et al., 1996]. Next, *CDH6* as well as *CDH1*, both members of the cadherine superfamily, are thought to contribute to progression in cancer by enhancing proliferation, invasion, and metastasis. The E-cadherine gene *CDH1* has been related to many cancers [Risinger et al., 1994]. The underlying mechanisms for the impaired E-cadherin expression in ovarian cancer, however, have not been completely determined. Based on Risinger's report, somatic mutations in *CDH1* are rare in ovarian cancer, suggesting that other mechanisms of inactivation may be involved. We confirm with our findings that *CDH1*, as part of a pathway, is downregulated in the group of sporadic ovarian cancers.

On the contrary, the tumors in the *BRCA1 group* seem to act through (in)activation of a distinct group of genes and pathways. More specifically the HOX genes, the metalloproteinases (MMPs), the tumor suppressor genes and the genes implicated in the estrogen signaling pathway are aberrated. The HOX pathway shows a complex mode of aberration. A part of this pathway is upregulated, whereas another part is downregulated. The HOX Homeobox proteins are transcription factors involved in growth control and differentiation during embryogenesis as well as homeostasis. When deregulated, they play an important role in oncogenesis [Grier et al., 2005]. Although recent studies have ascertained a role of some of these homeobox genes in ovarian cancer [Crijns et al., 2006], the order in which genomic aberrations and HOX gene expression changes occur is unclear. Furthermore, the processes driving aberrant HOX expression remain completely unknown. It is plausible that prolonged exposure of ovarian superficial epithelial cells to sex steroids throughout adult reproductive female life contributes to inappropriate HOX activation [Hennessy and Mills, 2006]. Our results give a first indication of which genes out of this group are gained or lost.

The MMP pathway is upregulated in the BRCA1 group. The MMPs are implicated in the breakdown of extracellular matrix and are believed to have a role in tumor initiation and metastasis. A part of the cluster is localized on chromosome 11q22.3, and this locus has been described before by Zweemer et al. [2001] as a location of alterations distinctive for familial ovarian cancer.

As shown clearly, a significant part of the estrogen signaling pathway is lost in the BRCA1-mutated ovarian tumors. Although the majority of epithelial ovarian cancers (40–60%) express estrogen receptors (ERs), only a small proportion (7–18%) of patients respond to antiestrogen treatment and thus uncertainty remains about the prognostic value of the ER status, its expression, and promoter methylation [Cunat et al., 2004]. Decreased ER-α expression has been described in malignant ovarian cells in comparison with human ovarian surface epithelial cells [Lau et al., 1999], whereas others did not confirm this but cited either the ratio of ER-α/ER-β to be determining for the change to neoplastic growth [Lindgren et al., 2004]. *CARM1* (MIM# 603934) is a positive regulator of ER-α-mediated transcriptional activation, and is essential for estrogen-induced expression of the critical cell cycle transcriptional regulator *E2F1* (MIM# 189971). This gene can

therefore be a critical factor in the pathway of ovarian cancer similar as in estrogen stimulated breast cancer growth [Frietze et al., 2008]. Both pathways, lost in our BRCA1-mutated tumors, could therefore be closely related, and be a possible explanation for the tissue specificity of the familial breast and ovarian cancers contributed to BRCA mutations, as already suggested by Fan et al. [1999]. Moreover, this suggests the ER and CARM pathway to be potential new targets in the treatment of BRCA related ovarian cancer. Furthermore, Hua et al. [2008] described in vitro studies, suggesting estrogens to promote cell migration and invasion by activating the PIK3/AKT pathway. This latter pathway is recently subject to many studies and recognized as a key pathway in ovarian cancer carcinogenesis and a potential target for therapy. Also, *KLK5* (MIM# 605643), a member of the kallikrein and the estrogen pathway, is implicated in carcinogenesis and cancer progression and is upregulated by estrogens. Some members of the kallikreines have potential as novel cancer biomarkers; for example, *KLK 5*, *6*, *7*, *10*, *11*, and *14* (respectively MIM#s 605643, 602652, 604438, 602673, 604434, and 606135) [Paliouras et al., 2007]. Next, the well-known *TP53*, also a member of this pathway, encodes for tumor protein p53 and regulates target genes that induce cell arrest, apoptosis, senescence, and DNA repair. Downregulation of this gene, functioning as a tumor suppressor gene, has been associated with many tumors. Almost 80% of ovarian tumors in patients with BRCA1 mutations harbor p53 mutations [Buller et al., 2001]. It is believed that for BRCA1 inactivation to contribute to malignant progression, p53 has to be inactivated as well and before the inactivation of the second BRCA1 allele [Evers and Jonkers, 2006; Prat et al., 2005]. By demonstrating the presence of both in a same pathway, we confirm this hypothesis with our results.

Other genes in the estrogen signaling pathway have been designated as being important in angiogenesis and cell cycle control, as do *THBS2* (MIM# 188061) and *TNFAIP2* (MIM# 603300), respectively, and *SPRR1B* (MIM# 182266), cycline D1 (*CCND1*; MIM# 168461), and *CDKN1B* (MIM# 600778). Cycline D1 overexpression is reported in variable proportion of ovarian tumors, but amplification is far less common [Dhar et al., 1999]. Most tumors expressing *CCND1* are low grade, whereas BRCA related tumors are often high grade and here we demonstrate the pathway involving *cyclin D1* to be lost.

Finally, another pathway different between both groups is the regulatory pathway, containing the tumor suppressor genes. These genes control the order and timing of cell cycle transitions to ensure correct DNA replication and chromosome segregation. Disruption of this pathway has been described to be related to the prognosis of some cancers and the role of *CDKN2A* and *RB1* expression herein has been described; this could not been demonstrated for *CCND1* [Song et al., 2008].

Small sample size is a severe problem when looking at genetic alterations in tumors seen in their individual heterogeneity and instability. It affects the statistical reliability of the conclusions. We concede that our study group is small; however, our results indicate significant differences between both groups of patients and these results are an important knowledge base for further investigation using a larger group of patients in the future to confirm our findings.

In conclusion, this aCGH pilot study demonstrates that different oncogenetic pathways are active in sporadic and BRCA1-related ovarian cancers. Larger studies are compulsory to confirm our findings. If confirmed, this knowledge will guide us in the search for new and specific targets in prevention and/or treatment of hereditary ovarian cancer.

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