Chromosome 19 Rearrangements in Ovarian Carcinomas: Zinc Finger Genes Are Particularly Targeted

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Chromosome 19 is frequently rearranged in ovarian carcinomas, but the pathogenetic consequences of this are not clearly understood. We performed microarray gene expression analysis on 12 ovarian carcinomas that carry a rearranged chromosome 19 in their karyotype. These aberrant chromosomes have previously been microdissected and analyzed by array-based CGH. In the current study, we wanted to explore whether the genomic alterations thus detected correlated with changes in gene expression. The microarray gene expression analysis gave information on 407 genes mapping in gained genomic regions on chromosome 19, of which 92 showed association between DNA gain and upregulated expression. Of the genes showing this association, 39 (42%) showed gain in at least two samples. The majority of these 39 genes of interest ($n = 24$, 62%) encode zinc finger proteins, which otherwise make up only 15% of the approximately 1,400 genes on chromosome 19. The strongest association was found for *ZNF223* which was upregulated in samples with genomic gain compared with samples without gain. We suggest that DNA copy number changes brought about by rearrangements of chromosome 19 contribute to ovarian carcinogenesis by leading to upregulation of *ZNF223* and other zinc finger genes.

INTRODUCTION

Cancer of the ovary accounts for 30% of all cancers of the female genital organs with an estimated 225,500 new cases worldwide each year (Jemal et al., 2011). Ovarian cancer is a diverse group of diseases with remarkable heterogeneity at the cellular and molecular levels, but with the epithelial subgroup (ovarian carcinomas) constituting 90% of primary malignant ovarian tumors (Bast et al., 2009). Ovarian carcinomas are known to be cytogenetically complex and genetically unstable (Gorringe and Campbell, 2009). Alterations of chromosome 19, in particular 19p13, are among the most frequent structural aberrations (Pejovic et al., 1989, 1992; Jenkins et al., 1993; Taetle et al., 1999). 19p and/or 19q are rearranged in 23% of the 457 ovarian carcinomas reported with karyotypic aberrations (Mitelman et al., 2013), with the rearrangements mostly being described as add(19p) and/or add(19q). The frequent occurrence of chromosome 19 rearrangements indicates that they are important in ovarian carcinogenesis. Due to the small size of this chromosome and its homogeneous staining by G-banding, chromosome 19 rearrangements can easily be mistaken for markers of unknown origin in the karyotype, thus the true prevalence of tumors with a rearranged chromosome 19 may be even higher than what is

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

Supported by: Norwegian Cancer Society, the South-East Norway Regional Health Authority, the Inger and John Fredriksen Foundation for Ovarian Cancer Research, and the Research Council of Norway through its Centres of Excellence funding scheme; Grant number: 179571.

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Received 18 October 2013; Accepted 4 March 2013

DOI 10.1002/gcc.22166

Published online 14 March 2014 in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. Ovarian Carcinomas with Chromosome 19 Rearrangements Included in the Study

Sample	Histologic subtype[a]	FIGO stage	No. genes with gain[b]	No. genes with association[c]
1	HGSA	IIIB	89	15
2	HGSA	IIIC	3	0
3	HGSA	IIIC	25	3
4	HGSA	IIIC	42	3
5	HGSA	IV	97	28
6	HGSA	IV	124	28
7	HGSA	IIIC	113	30
8	UC[d]	IIC	1	0
9[e]	HGSA	IIIC	77	13
10	HGSA	IIIC	129	42
11	HGSA[d]	IIC	33	6
12	HGSA	IIIC	1	0

[a]HGSA =, high high-grade serous adenocarcinoma,; UC =, undifferentiated carcinoma.
[b]No. of gene loci with DNA gain identified by aCGH (Micci et al., 2010).
[c]No. of genes showing association between DNA gain and upregulated gene expression (Table 1 and Supporting Information Table S1).
[d]Additional histochemistry was performed to get the final diagnosis.
[e]Patient received neoadjuvant chemotherapy.

presently reported. Also in studies using molecular methods, copy number alterations of this particular chromosome have been frequently found (Nakayama et al., 2007). In The Cancer Genome Atlas (TCGA) data set for ovarian cancer (Cancer Genome Atlas Research Network, 2011), 5 of the 20 most significantly amplified regions are found on chromosome 19, among them the *CCNE1* locus at 19q12. It has been suggested that DNA copy number alteration may be an early event in ovarian carcinogenesis and a primary determinant of altered gene expression in high-grade serous ovarian carcinomas (Bowtell, 2010).

Our group has previously studied 26 primary ovarian carcinomas selected because of the presence of an add(19p) and/or add(19q) marker(s), among other abnormalities, in their karyotypes. To evaluate the DNA composition of the abnormal chromosomes, we first performed microdissection to isolate the markers of interest and then did reverse in situ hybridization (rev-ish) to determine which chromosomes the genetic material came from (Micci et al., 2009). DNA from each microdissected marker was also amplified and hybridized onto microarrays using a comparative genomic hybridization (CGH) protocol and areas with gained material were identified (Micci et al., 2010). The DNA copy number analysis was thus performed on isolated aberrant chromosomes that are non-randomly observed in ovarian carcinomas and which, consequently, are likely to be pathogenetically important. For convenience, the copy number analysis of the microdissected DNA material will be referred to as "aCGH." Genes within the loci on chromosome 19 identified as gained will be referred to as "genes with DNA gain." These genes have been subjected to genomic rearrangements and are thus of interest for our understanding of ovarian carcinogenesis as they may be active in the process of tumorigenesis and/or tumor progression.

The aim of the present study was to investigate further the nonrandom nature of chromosome 19 rearrangements in ovarian carcinomas by investigating the relationship between genomic alterations and gene expression.

MATERIALS AND METHODS

Tumor Samples

The tumors have been previously examined by us in studies using karyotyping, rev-ish, and aCGH (Micci et al., 2009, 2010) and were all surgically removed at The Norwegian Radium Hospital between 1999 and 2004. The 12 high-grade carcinomas (11 serous and 1 undifferentiated) included in the present study were originally selected because of the presence of one or several structurally rearranged copies of chromosome 19 in the karyotype (Table 1). All samples from the original cohort ($n = 203$) with a rearranged chromosome 19 and available material for further studies were included in the present study ($n = 12$). The frequency of chromosome 19 rearrangements in the original cohort was 13% ($n = 26$). Regions of gain for each sample, as identified by aCGH, are provided in Supporting Information Table 1.

Tumor histology and grade have been revised by a gynecological pathologist. The tumor biobank

has been registered according to national legislation and the study has been approved by the Regional Committee for Medical Research Ethics South-East in Norway.

Isolation of RNA

Tumor tissue adjacent to that used for cytogenetic analysis and histologic examination had been frozen and stored at −80°C. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and miRNAeasy spin columns (Qiagen GmbH, Hilden, Germany). First, tumor tissue was homogenized in TRIzol and the aqueous phase was removed and processed with the Qiagen miRNeasy Mini kit according to the manufacturer's protocol. Total RNA was quantified and the purity was assessed using a NanoVue spectrophotometer (GE Healthcare, Little Chalfont, UK). RNA quality was checked by the Experion automated electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA). All 12 samples had RQI (RNA quality indicator) value 6.7 or higher.

Microarray Gene Expression Analysis

One hundred nanograms of total RNA were used as input for global gene expression analysis using Affymetrix GeneChip Human Exon 1.0 ST Arrays (Affymetrix, Santa Clara, CA, USA). Each microarray contains 1.4 million probe sets (the majority of which are comprised of four probes), where each probe set corresponds to approximately one exon. A total of 289,961 probe sets target well annotated full-length human mRNAs ("core" probe sets; GeneChip Exon Array Design, http://www.affymetrix.com/support/technical/technotes/exon_array_design_technote.pdf). RNA from each sample was individually amplified, reversely transcribed, fragmented, and labeled (The Ambion WT Expression Kit, http://media.affymetrix.com/support/downloads/manuals/wt_expressionkit_manual.pdf). Labeled sense strand cDNA was hybridized onto the microarrays for 16–18 hr (GeneChip WT Terminal Labeling and Hybridization User Manual, http://www.medsci.uu.se/digitalAssets/85/85484_wt_term_label_ambion_user_manual.pdf). The microarrays were washed, stained, and scanned according to the manufacturer's protocol.

Cell intensity (CEL)-files from the 12 tumor samples were background corrected, inter-chip quantile normalized, and summarized at gene level by the robust multi-array average (RMA) approach (Irizarry et al., 2003) implemented in the Affymetrix Expression Console 1.1 software. Using the HuEx-1_0-st-v2.r2 core library files and the HuEx-1_0-st-v2.na33.hg19.transcript.csv annotation file, 17,361 genes (i.e., transcript clusters annotated with gene symbols) were identified.

A normal ovary sample (RNA extracted from whole ovary; Cat#HR-406 Human Ovary Total RNA, Zyagen, San Diego, CA, USA) was analyzed in three separate runs, normalized with the tumor samples, and used for comparison of technical variation with the cancer samples and to evaluate the gene expression of previously known ovarian carcinoma-related genes.

Integration of DNA Copy Number Gains and Gene Expression Data

Data from the aCGH analysis were originally annotated according to the Human Genome 18 (hg18) NCBI36 (Micci et al., 2010). To enable integration of the two data sets, the aCGH data were converted to the Human Genome 19 (hg19) GRCh37 using the LiftOver tool at UCSC (genome.ucsc.edu/cgi-bin/hgLiftOver).

To identify genes of interest, RNA expression data were integrated with DNA copy number data from the microdissected chromosome 19 markers. A computer script was used to integrate the normalized expression values for each sample with the corresponding aCGH findings on a gene-by-gene and locus-by-locus basis across chromosome 19, as previously described by Brekke et al. (2010). To compare with random expression values, permutation analysis with 10 repetitions was performed; the expression data for each gene was shuffled across the samples and the integration analysis was repeated.

RESULTS

Identification of 39 Genes of Interest

In the gene expression data, 1,211 genes were annotated with gene symbols on chromosome 19. Analyzing the aCGH data (Micci et al., 2010) and the gene expression data from the same tumors, we identified 407 genes in regions with DNA gain. To analyze association between DNA gain and gene expression, we used the samples as mutual controls. This could be done since no single genomic locus was gained in all samples. The median gene expression value in samples with genomic gains was compared to samples without

TABLE 2. Genes Showing Association Between DNA Gain and Gene Expression

Gene[a]	Ratio_Gain/ NoGain[b]	ZNF cluster[c]	Samples with gain[d]	Gene start (bp)	Gene end (bp)	Cytoband	mRNA accession
ZNF223	2.67	3	7,10	44 556 164	44 576 275	19q13	NM_013361
SLC44A2	2.50		5,6	10 713 140	10 755 225	19p13	NM_001145056
HIF3A	2.38		7,10	46 800 334	46 846 681	19q13	NM_152794
ZNF627	2.27	1	5,6,9	11 696 045	11 729 939	19p13	NM_145295
ZNF681	2.12	2	1,6,7,9,11	23 926 170	23 945 122	19p12	NM_138286
ZNF285 // ZFP112	2.04	3	7,10	44 873 109	44 916 796	19q13	AK302594
TECR	2.02		5,6,7	14 627 312	14 676 786	19p13	NM_138501
SMG9	2.00		7,10	44 235 312	44 259 821	19q13	NM_019108
ZNF230	1.98	3	7,10	44 507 004	44 518 062	19q13	NM_006300
ZNF226	1.98	3	7,10	44 669 237	44 682 524	19q13	NM_001032372
LOC147727	1.94		5,6	10 762 548	10 764 998	19p13	NR_024333
ZNF43	1.91	2	6,7	21 987 768	22 034 838	19p13-p12	NM_003423
NOTCH3	1.89		5,7	15 263 493	15 311 792	19p13	NM_000435
KEAP1	1.88		5,6	10 596 571	10 614 233	19p13	NM_203500
NR2C2AP	1.80		7,9	19 312 232	19 318 278	19p13	NM_176880
ZNF45	1.78	3	7,10	44 416 797	44 439 411	19q13	NM_003425
ZNF737	1.76	2	1,3,6,7,9	20 451 078	20 849 839	19p12	NM_001159293
ZNF433	1.75	1	1,5,6,10	11 832 081	12 146 515	19p13	NM_001080411
ZNF430	1.75	2	1,3,6,7,9	21 168 360	21 309 542	19p12	NM_025189
ZNF91	1.74	2	1,6,7,9,11	23 542 205	23 701 860	19p12	NM_003430
OR7A17	1.73		6,7	14 991 238	14 992 167	19p13	NM_030901
ZNF675	1.68	2	1,6,7,9,11	23 708 433	23 869 994	19p12	NM_138330
ZNF225	1.64	3	7,10	44 617 541	44 637 810	19q13	NM_013362
ZNF441	1.64	1	1,5,6,10	11 877 907	11 894 883	19p13	NM_152355
ZNF100	1.61	2	6,7	21 905 568	21 950 368	19p12	NM_173531
ZNF729	1.61	2	7,9	21 991 874	23 041 235	19p12	NM_020855
AKAP8	1.60		5,6,7	15 464 216	15 490 603	19p13	NM_005858
ELOF1	1.60		5,6,9	11 661 972	11 670 131	19p13	NM_032377
ILF3	1.59		5,6	10 764 547	10 803 074	19p13	NM_012218
ZNF440	1.57	1	1,5,6,10	11 925 107	11 946 414	19p13	NM_152357
QTRT1	1.56		5,6	10 812 137	10 824 040	19p13	NM_031209
ZNF431	1.55	2	4,6,7,11	21 324 837	21 519 513	19p12	NM_133473
RNASEH2A	1.55		5,6	12 912 886	12 924 462	19p13	NM_006397
RPSAP58	1.54		1,6,7,9,11	23 317 531	24 011 650	19p12	NR_003662
ZNF155	1.54	3	4,7,10	44 488 355	44 502 469	19q13	AF187986
ZNF208	1.53	2	7,9	22 035 235	22 193 735	19p12	NM_007153
ZNF700	1.51	1	5,6	11 750 601	12 091 412	19p13	NM_144566
ZNF439	1.50	1	1,5,6,10	11 959 589	11 994 545	19p13	NM_152262
ZNF93	1.50	2	1,3,6,7,9,11	19 944 282	20 237 821	19p12	NM_031218

[a]Gene symbols according to Human Genome 19 (hg19) GRCh37.
[b]Association: Ratio Gain/NoGain = (median expression samples with gain)/(median expression samples without gain).
[c]Cluster of KRAB-Zinc finger genes according to Fig. 1A.
[d]Samples with DNA gain in gene locus identified by aCGH (Micci et al., 2010).

gains for each gene. The ratio between the two groups (=median expression Gain/median expression NoGain) was used to rank the genes according to degree of association between genomic gain and elevated expression. An association was found in 92 genes (ratio Gain/NoGain ≥ 1.5). We excluded genes that showed genomic gain in only one sample to minimize the possibility of unspecific findings (Supporting Information Table 2), thus narrowing down the list of genes with association between overexpression and DNA gains to 39 genes (Table 2). We also tested the use of average expression values instead of median expression values, getting similar results. We did not perform further statistical analysis due to the small sample size.

Overrepresentation of the Zinc Finger Gene Family

A total of 24 of the 39 (62%) genes showing association between expression and gain in at least two samples belong to the zinc finger gene family (Table 2). The zinc finger (ZNF) genes with

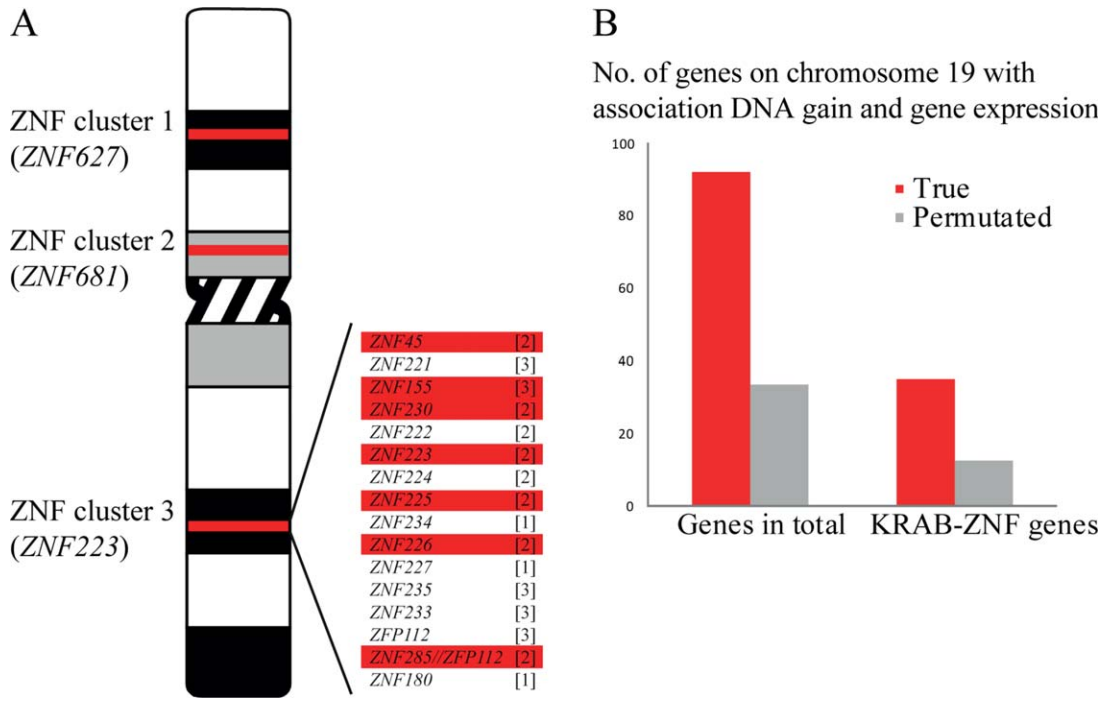

Figure 1. Genes with association between DNA gain and upregulated gene expression on chromosome 19. (A) The strongest association was found for zinc finger (ZNF) genes residing in three clusters (the gene showing strongest association in each cluster noted in parenthesis). ZNF cluster 3 shown in detail, genes with association between DNA gain and upregulated gene expression in at least two samples are marked in red. Number of samples with DNA gain in each gene locus is noted in brackets. (B) Permutation of data. In the true data set (red), association between DNA gain and upregulated gene expression is more frequent than in the permutated data (gray), with association in 93 and 33.5 genes, respectively. Association is especially common in zinc finger genes in the true data set compared with the permutated data set (35 and 12.5 genes, respectively).

association cluster to three genomic regions (Fig. 1A), further referred to as ZNF cluster 1 (from 11.69 Mbp to 12.72 Mbp), ZNF cluster 2 (from 19.77 Mbp to 24.31 Mbp), and ZNF cluster 3 (from 44.41 Mbp to 45.00 Mbp). In 10 of the 12 samples, there were DNA gains in at least one of these clusters (Table 2). The degree of association between DNA gain and gene expression varied within each cluster, but each cluster had one gene that showed a markedly stronger association than the rest: zinc finger protein 627 gene (*ZNF627*), zinc finger protein 681 gene (*ZNF681*), and zinc finger protein 223 gene (*ZNF223*), respectively (Fig. 1A), and seven of the samples showed association between DNA gain and gene expression in at least one of these genes. Permutation analysis (Fig. 1B), performed with shuffled gene expression data, yielded data sets with fewer genes with association between expression and DNA gain (median 33.5, range 24–41 genes) and a lower proportion of zinc finger genes with association (median 12.5, range 5–18) than in the true data set (92 genes with association, of which 35 are zinc finger genes). Zinc finger genes constitute 15% of the genes on chromosome 19, but we found this gene family to be overrepresented among genes with association between DNA gain and upregulated gene expression compared to other genes (38% zinc finger genes [$n = 35$] vs. 62% other genes [$n = 57$]; $P < 0.001$, Fisher exact test; Table 3). The permutation analysis supports this finding. These frequencies could possibly be higher since the total RNA was extracted from samples with a mixture of tumor cells and stromal components.

The strongest association was found for *ZNF223* in ZNF cluster 3. For *ZNF223*, the gene expression in the samples with gains (samples 7 and 10) was more than two-fold higher compared to the other samples (ratio Gain/NoGain = 2.67, Fig. 2). In the same cluster, eight other zinc finger genes also show association between gain and expression (Table 2 and Supporting Information Table 1) while seven show low association. Interestingly, *ZNF230* (ratio Gain/NoGain = 1.98, Fig. 2) showed a similar expression profile to *ZNF223*, with upregulated gene expression and gain in the same samples.

In ZNF cluster 1, six zinc finger genes showed association between gain and expression (Table 2), most pronounced for *ZNF627* (ratio Gain/

TABLE 3. Overrepresentation of Zinc Finger Genes

Cys_2His_2 zinc finger genes with KRAB domain[a]	Proportion of genes
Total genome	~2%
Chromosome 19	~15%
DNA copy number gain (103/407)	25%
Association gain and expression (35/92)	38%
Association gain and expression, ≥2 samples (24/39)	62%

[a]According to HUGO Gene Nomenclature Committee (www.genenames.org) and Universal Protein Resource (www.uniprot.org).

NoGain = 2.27, Fig. 2). Also 12 other zinc finger genes are annotated to this cluster in our data but for these genes we did not find association between DNA gain and upregulation of gene expression.

ZNF681 (ratio Gain/NoGain = 2.12, Fig. 2) showed the strongest association between DNA gain and gene expression in ZNF cluster 2. This cluster is the largest of the three, with 22 informative zinc finger genes of which 11 showed association in at least two tumors.

The solute carrier family 44 member 2 gene (*SLC44A2)* gave the second highest association value in total (ratio Gain/NoGain = 2.50, Fig. 2). Gene expression was clearly upregulated in the two samples with DNA gain, whereas nearly all the samples without gain had lower expression values. Interestingly, sample 4 showed no DNA gain in its locus but gene expression was similar to the genes with gain.

Comparison with Data from TCGA

Our results are based on the analysis of 12 ovarian carcinomas, selected because of chromosome 19 rearrangements, from a series of 203 ovarian carcinomas karyotyped by our group (data not shown). To evaluate the prevalence of amplification and upregulated gene expression in a larger series, we compared our findings with the TCGA ovarian cancer data set (data on mRNA expression, DNA copy number alteration, and DNA sequence from 316 ovarian serous adenocarcinomas, available online: www.cbioportal.org/public-portal, default settings). For *ZNF223*, 67% of the samples with amplification had overexpression, while only 2% of the samples without amplification show overexpression. Concurrent DNA gain and RNA upregulation of *ZNF223*, *ZNF627*, *ZNF681*, and *SLC44A2* were found in 1.3%, 1.9%, 4.4%, and 1.9%, respectively, of the serous ovarian carcinomas in TCGA (for frequency of amplification and overexpression, see Supporting Information Table 3). The regions of amplification on chromosome 19 identified in the TCGA cohort (as reported in their Supporting Information Table 5.2 (Cancer Genome Atlas Research Network, 2011) have been compared to the regions of gains in our samples, and the data are reported in Supporting Information Table 1.

Findings in Known Ovarian Carcinoma-Related Genes

Several commonly amplified genes in ovarian carcinomas showed varying results; the Neurogenic locus notch homolog protein 3 gene (*NOTCH3*) showed association between DNA gain and gene expression (ratio Gain/NoGain = 1.89, Table 2), while the cyclin E1 gene (*CCNE1*) and the URI1, prefoldin-like chaperone gene (*URI1*) both had DNA gain in one sample but showed no association. The v-akt murine thymoma viral oncogene homolog 2 gene (*AKT2*) did not have DNA gain in any sample (for gene expression levels and DNA copy number results in these and in other ovarian cancer-related genes that did not rank among our genes of interest, see Supporting Information Fig. 1).

In all samples, the kallikrein genes *KLK6*, *KLK7*, and *KLK8* were among the most markedly overexpressed genes, while the paternally expressed 3 gene (*PEG3*) was the most downregulated, when comparing to expression in the normal control. However, no samples had DNA copy number gain of these loci.

DISCUSSION

We have analyzed ovarian carcinomas with structurally rearranged copies of chromosome 19 by comparing DNA copy numbers and gene expression. We focused on chromosome 19 because of its nonrandom involvement in ovarian carcinomas (Pejovic et al., 1992; Taetle et al., 1999). DNA copy number variation has the potential to amplify oncogenes and/or result in loss of tumor suppressor genes in many types of cancer (Zhao et al., 2004; Beroukhim et al., 2010), including ovarian carcinomas (Gorringe et al., 2010). Earlier studies have found that DNA copy number variation can explain almost 20% of gene expression variation (Stranger et al., 2007). It has also been suggested that correlation between gene expression and DNA copy number might be unusually high in ovarian carcinomas compared

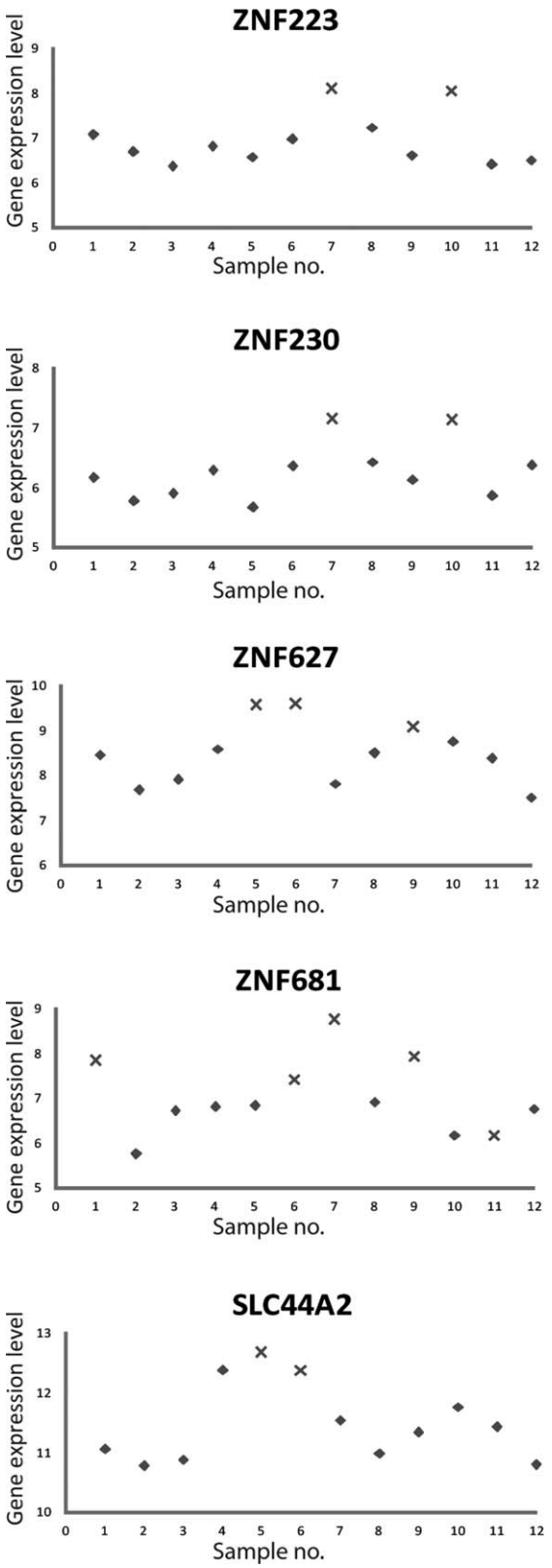

Figure 2. Gene expression levels (*y*-axis, values in $\log_2$) in 12 ovarian carcinomas (*x*-axis) for five selected genes with association between DNA gain and upregulated gene expression. Samples marked with "X" have DNA gain in the gene locus.

with other solid tumors (Bowtell, 2010). We found 39 genes residing in regions that were gained through chromosome 19 rearrangements which showed upregulated expression in at least two samples (Table 2).

Chromosome 19 harbors a number of clustered gene families. The zinc finger gene family is the most numerous and has been shown to cluster to 11 genomic regions (Grimwood et al., 2004). The majority of the genes with association between DNA gain and gene expression in the present study (24 of 39 genes) reside in three of these clusters and encode the subgroup of zinc finger proteins that contain the Krüppel associated box (KRAB) domain (Fig. 1A). The KRAB-containing zinc finger proteins represent the largest group of transcription factors encoded by the human genome (Corsinotti et al., 2013) and make up 15% of all protein coding genes on chromosome 19 according to the HUGO Gene Nomenclature Committee (HGNC; www.genenames.org). The permutation analysis supports the notion that gene expression is non-randomly associated with DNA copy number in zinc finger genes (Fig. 1B).

Each of the three zinc finger clusters contains one gene that shows a stronger association than the rest. The strongest association was found for *ZNF223*, where samples with DNA gain showed a clear upregulation compared with samples without gain. The *ZNF223* gene encodes a zinc finger protein with 10 Cys_2His_2 domains (so-called "classic" zinc finger domains, also known as Krüppel-type zinc fingers) in addition to one KRAB domain (www.uniprot.org). The specific target gene(s) of *ZNF223* are unknown. The KRAB domain is considered to be a transcriptional repressor and has been suggested to repress RNA polymerase 1, 2, and 3 promoters (Urrutia, 2003), but the specific functions of individual KRAB-containing zinc finger proteins remains mostly unknown (Corsinotti et al., 2013). Six other zinc finger genes in the same cluster (Fig. 1A, Table 2) showed association between DNA gains and upregulated gene expression in at least two samples. These genes are paralog genes according to GeneCards (www.genecards.org). Transcription factors that share sequence similarity might still have different effects on regulatory function if the nucleotide sequence difference affects the DNA affinity (Vaquerizas et al., 2009). *ZNF230*, residing in the same cluster, has the same two samples showing concurrent gain and upregulation as *ZNF223* (Fig. 2). Generally, the zinc finger genes are expressed at low levels, and it is known that transcription

factors tend to be expressed at low levels (Ghaemmaghami et al., 2003).

Some of the zinc finger genes in the two other clusters showed association between DNA gain and gene expression (Fig. 1A), especially *ZNF627* (ZNF cluster 1) and *ZNF681* (ZNF cluster 2). The genomic region of ZNF cluster 2 was identified as commonly gained in several of the samples in the aCGH study from the same material (Micci et al., 2010), as well as in the TCGA cohort (Cancer Genome Atlas Research Network, 2011). In the present study, this was the most commonly gained area (8 of 12 samples). Little is known about the biological function of these zinc finger genes, but interestingly, *ZNF681* and other genes in ZNF cluster 2 are suggested to play a functional role in modulating response to tyrosine kinase inhibitors in patients with gastrointestinal stromal tumors (Rink et al., 2013).

The regulation of zinc finger gene expression is not fully understood. Our results suggest that DNA copy number gain is a mechanism for gene expression upregulation. Others have also found that overexpression can been linked to DNA amplification (Yang et al., 2011), but epigenetic mechanisms and autoregulatory mechanisms also seem to be a part of zinc finger gene expression control (O'Geen et al., 2007; Yu et al., 2013). Individual KRAB-containing zinc finger genes residing in the same genomic cluster exhibit distinctive modes of expression (Huntley et al., 2006; Corsinotti et al., 2013), and this is supported by the present results. The variable expression of closely situated genes indicates individual roles and functions, even if they show sequence similarities.

The relationship between DNA gain and upregulated gene expression is not linear, as demonstrated by *SLC44A2*, where DNA copy number gain did not seem to affect gene expression in one of the samples (Fig. 2).

Genomic rearrangements can result in the creation of fusion genes. In epithelial cancers, fusion partners commonly encode tyrosine kinase receptors and transcription factors (Prensner and Chinnaiyan, 2009). From exon-level expression profiles, we found no indications of fusion genes in the examined samples (data not shown); however, it is interesting that transcription factors are overrepresented among genes with association between DNA gain and gene expression. DNA rearrangements may also result in deregulation of gene expression (Mitelman, 2000) if a promoter region of one gene is fused to another gene's coding region as in the deregulation of *MYC* in Burkitt's lymphoma. This mechanism might have played a role in the upregulated gene expression in our samples; however, this is not visible from exon-level expression profiles, and we did not pursue the hypothesis further.

Sequence homology may be a source of cross-hybridization and thus give uncertain data in microarray analyses. It could be argued that this might be the case for the similarity observed among the zinc finger family members. However, the oligo design is optimized to target unique sequences for each individual gene (and exon). The fact that, in general, different samples had overexpression of different zinc finger genes, speaks in favor of reliable measurements.

In the present study, we evaluated the effect of gains of genomic material and did not consider losses. The aCGH study was based on analyses of microdissected chromosomes, thus we have no information on whether loci not present on the marker chromosomes actually were lost from the cancer genomes (Micci et al., 2010).

Several zinc finger genes have been linked to malignancies, also to ovarian cancer (Sun et al., 2008; Feng et al., 2008; Elgaaen et al., 2012). In the Cancer Gene Census (a catalog of genes for which mutations have been causally implicated in cancer, http://cancer.sanger.ac.uk/cancergenome/projects/census/), *ZNF331* is the only listed zinc finger gene mapping to chromosome 19. This putative tumor suppressor, suggested to have a repressing effect on cell proliferation, is significantly downregulated in gastric cancer cells (Yu et al., 2013). In the present study, none of the tumor samples had DNA copy number gain of *ZNF331*.

Our results are based on the analysis of a limited series of 12 tumors, but association between upregulation of mRNA and genomic gain of *ZNF223*, *ZNF627*, *ZNF681*, and *SLC44A2* is also present in the data from the TCGA data set of serous ovarian carcinomas. In a study of the TCGA data set, Wrzeszczynski et al. (2011) examined the correlation between gene expression and DNA copy number variation genome-wide and identified 54 genes thus associated, 35 of which (65%) map to chromosome 19. However, none of them belong to the family of KRAB-zinc finger genes that stood out in our data. In another study of this data set, Shih et al. (2011) selected 100 possible "driver" oncogenes in ovarian carcinomas by analyzing the correlation between DNA copy number and mRNA expression and found that 54 of them mapped to chromosome 19. Seven zinc

finger genes were among these suggested oncogenes, of which three also showed association in our data set, i.e., *ZNF585B*, *ZNF507*, and *ZNF383* (marked with * in Supporting Information Table 2).

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