Genetic profiles distinguish different types of hereditary ovarian cancer

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Abstract. Heredity represents the strongest risk factor for ovarian cancer with disease predisposing mutations identified in 15% of the tumors. With the aim to identify genetic classifiers for hereditary ovarian cancer, we profiled hereditary ovarian cancers linked to the hereditary breast and ovarian cancer (HBOC) syndrome and the hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Genome-wide array comparative genomic hybridization was applied to 12 HBOC associated tumors with *BRCA1* mutations and 8 HNPCC associated tumors with mismatch repair gene mutations with 24 sporadic ovarian cancers as a control group. Unsupervised cluster analysis identified two distinct subgroups related to genetic complexity. Sporadic and HBOC associated tumors had complex genetic profiles with an average 41% of the genome altered, whereas the mismatch repair defective tumors had stable genetic profiles, with an average 18% of the genome altered. Losses of 4q34, 13q12-q32 and 19p13 were overrepresented in the HBOC subset. Discriminating genes within these regions include *BRCA2*, *FOXO1A* and *RB1*. Gains on chromosomes 17 and 19 characterized the HNPCC tumors, but target genes herein are unknown. The results indicate that HBOC and HNPCC associated ovarian cancer develop along distinct genetic pathways and genetic profiles can thus be applied to distinguish between different types of hereditary ovarian cancer.

Introduction

Heredity is one of the strongest risk factors for ovarian cancer with 10-15% of the tumors linked to the hereditary breast and ovarian cancer (HBOC) syndrome and 2-4% to the hereditary non-polyposis colorectal cancer (HNPCC) syndrome (1-3). HBOC is caused by mutations in the *BRCA1* and *BRCA2* genes, with the highest (20-40%) life time risk of ovarian cancer in individuals with *BRCA1* mutations (4). HBOC associated ovarian cancer typically develops at 50-55 years of age with a predominance of serous adenocarcinomas (5). HNPCC is caused by defective mismatch repair (MMR), primarily due to mutations in the genes *MLH1*, *MSH2* and *MSH6*. Female HNPCC family members are at 10-15% risk of ovarian cancer. These tumors typically develop at an early age; mean 40-45 years, with a predilection for endometrioid, clear cell and mucinous subtypes (6,7).

Refined methods for risk prediction and early tumor detection are needed since late stage diagnosis constitutes a major reason for the poor prognosis of ovarian cancer.

The *BRCA1* and *BRCA2* genes, as well as the MMR genes, play critical roles in maintenance of genomic stability and repair. BRCA1 and BRCA2 are involved in double strand break repair, mainly through interaction with RAD51. BRCA1 inactivation evokes pleiotrophic effects on cell cycle control, transcriptional activation and consequently cells defective for BRCA1 exhibit extensive chromosomal instability (8). In ovarian cancer, the *BRCA1* genotype is linked to highly malignant tumors, in which no precancerous state has been identified (9). MMR defects interfere with repair of single mismatched base pairs and small nucleotide insertions/deletions. MMR defective tumors generally demonstrate few genetic changes and near diploid karyotypes, also in advanced tumor stages, though data on the genomic profiles of HNPCC associated ovarian cancer are scarce.

Identification of women with hereditary ovarian cancer allows for preventive measures for the more commonly occurring breast cancers in HBOC and colorectal and endometrial cancers in HNPCC (10,11). However, identification of these cases is challenging and a substantial number of individuals with hereditary ovarian cancer escape detection (12,13). We applied high resolution array based comparative genomic hybridization (aCGH) to HBOC and HNPCC associated ovarian cancer with sporadic ovarian cancers as a control group, and demonstrated distinct genetic profiles linked to the underlying type of heredity.

Materials and methods

Patients and tumors. Epithelial ovarian cancers (borderline tumors excluded) from 24 sporadic cases, 12 *BRCA1* mutated

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Table I. Summary of clinicopathological factors.

	HNPCC tumors n=8	HBOC tumors n=12	Sporadic tumors n=24
Age			
median (range)	47 (42-61)	57 (42-75)	60 (27-78)
FIGO stage			
I	3	1	3
II	3	1	1
III	1	8	15
IV	1	2	5
Histology			
Serous	1	8	17
Mucinous	1	0	3
Endometroid	3	3	4
Clear cell	3	1	0
Differentiation			
Well	1	1	5
Moderate	4	0	5
Poor	2	10	13
Unknown	1	1	1

cases and 8 cases with mutations in *MLH1*, *MSH2* and *MSH6* were available for the study. Clinical and histopathological data are summarized in Table I. A gynecological pathologist reviewed all diagnoses and four histotypes (serous, mucinous, endometrioid and clear cell adenocarcinomas) were represented (Table I). None of the patients had received preoperative radiotherapy or chemotherapy. The median age at diagnosis was 60 years in the sporadic subset, 57 years in the BRCA1 subset and 47 years in the HNPCC subset. The 24 sporadic tumors were obtained from a population based consecutive series of ovarian cancers, in which mutations in *BRCA1* and *BRCA2* had been excluded through mutation analysis and retained MMR function had been demonstrated using immunostaining against the MLH1, PMS2, MSH2 and MSH6 proteins (13,14). All *BRCA1* mutations were classified as disease predisposing and were available from a population based consecutive series of ovarian cancers. The MMR gene mutations were verified using immunohistochemistry and microsatellite analysis and disease predisposing mutations affected *MLH1* in one case, *MSH2* in three cases and *MSH6* in four cases. All ovarian cancers that had developed in Swedish females carrying HNPCC predisposing MMR gene mutations were eligible for the study. Further requirements for all tumors were availability of histopathological material and that DNA of sufficient amount and quality could be obtained. Ethical approval for the study was obtained from the Lund University ethics committee.

DNA extraction, labeling and hybridization. DNA was extracted from formalin-fixed, paraffin-embedded tumor tissue according to protocols from the UCSF Waldman Laboratory, San Francisco, CA, USA (http://cc.ucsf.edu/people/waldman/Protocols/paraffin.html), with an additional purification step using Phase Lock Gel tubes (Eppendorf AG, Hamburg, Germany). DNA quality was assessed using a Ready-To-Go RAPD analysis kit (GE Healthcare, Little Chalfont, UK) with primers 5'-AATCGGGCTG-3' and 5'-GAACGGGTG-3'. PCR products were validated on a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Tiling 32k BAC microarrays, with contiguous genome-wide coverage, were produced at the Microarray DNA Resource Centre, SCIBLU Genomics, Department of Oncology, Lund University, Sweden (http://www.lth.se/sciblu). Labeling and hybridization were performed as described by Jonsson *et al* (15). In short, 2-8 μg tumor DNA and 2 μg reference DNA (Promega Corp., Madison, WI, USA) were labeled with Cy3-dCTP and Cy5-dCTP, using BioPrime Array CGH Genomic Labeling System (Invitrogen Life Technologies, Carlsbad, CA, USA). Tumor and reference DNA were pooled and mixed with Human COT-1 DNA (Invitrogen). Hybridizations were conducted using the MAUI Hybridization System (BioMicro systems Inc., Salt Lake City, UT, USA) and the slides were scanned in an Agilent Microarray Scanner (Agilent Technologies).

Image processing and data analysis. Image analysis was carried out using the GenePix™ Pro. 4.1 software (Axon Instruments Inc., Foster City, CA, USA) and quantified data matrices were uploaded in the web based BioArray Software Environment software (BASE) (16). Positive and non-saturated spots were background corrected using the median background intensities for each channel and $\log_2$ ratios were calculated from the background corrected intensities. Data were filtered for flagged features and spots below signal to noise ratio 3 for both channels were eliminated. Data were normalized using an implementation of a population based LOWESS algorithm (17). Automatic breakpoint detection was performed using Circular Binary Segmentation (18). Copy number gains, losses and high level amplifications were determined by comparing segmented data to sample adaptive thresholds as described (17). The fraction of the genome altered was calculated as the total number of base pairs altered divided by the number of base pairs in the entire genome. Comparisons of data were made with regard to underlying mutation, histological type (serous vs. endometrioid, serous vs mucinous and endometrioid vs mucinous), grade (grade I and II vs. grade III and IV) and age at onset of disease (<40 years vs. >41 years) using MeV 4.1.01 software (http://www.tm4.org/mev.html) on segmented, logged values for permutation analysis. Further, unsupervised hierarchical clustering through the Pearson correlation distance metric was used (19). By applying significant testing for aberrant copy number (STAC) analysis to the sporadic, BRCA1 and MMR associated subgroups we determined the statistical difference in copy number alterations (20). Regions with a frequency or footprint p-value <0.05 were considered significantly altered. Copy number variants and altered telomeric and centromeric regions were excluded.

Microsatellite instability (MSI). MSI analysis was performed to ensure representative tumor DNA in the genetically stable

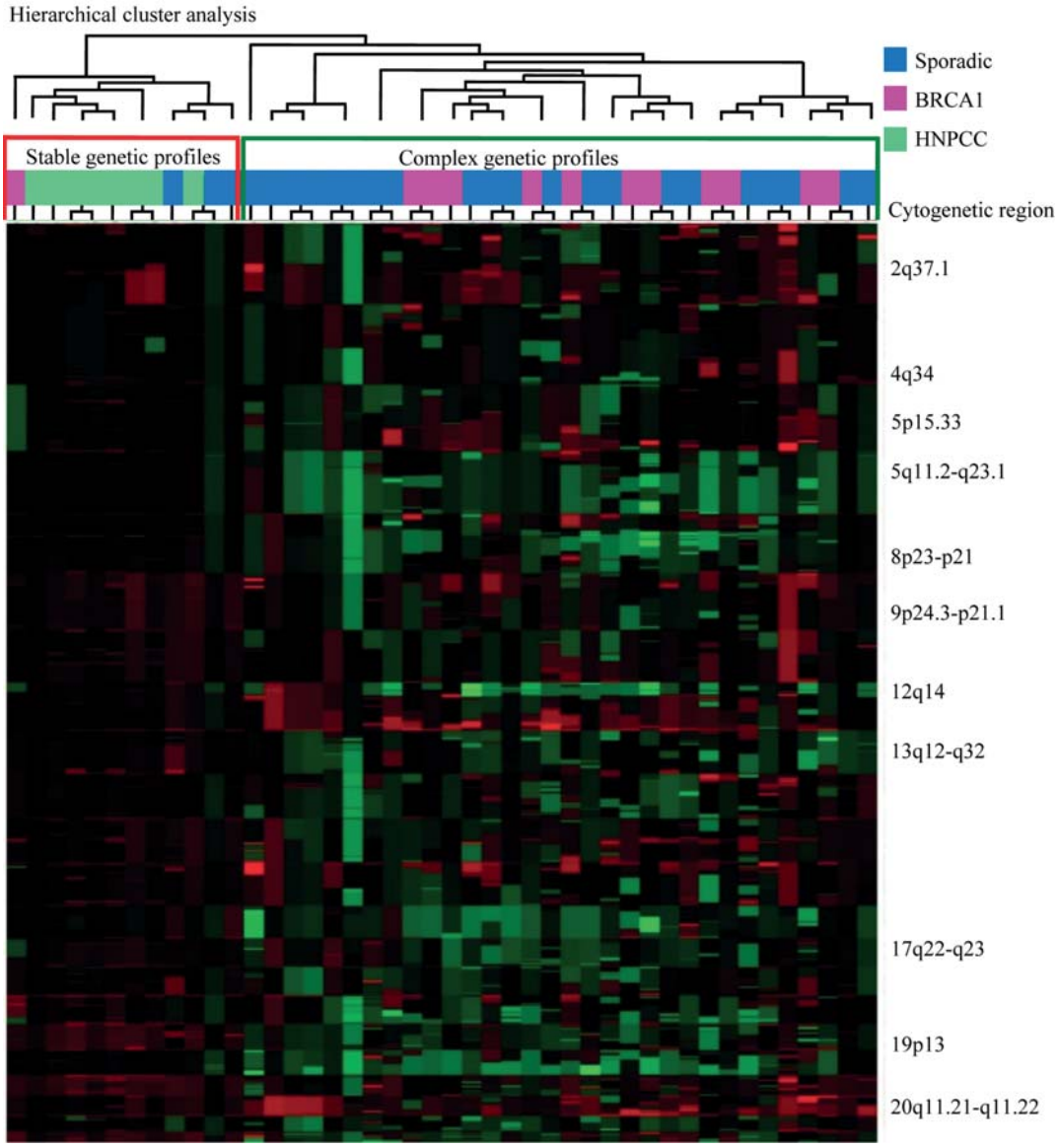

Figure 1. Unsupervised hierarchical cluster analysis of 12 BRCA1 mutated (pink), 8 MMR defective (green) and 24 sporadic (blue) ovarian tumors hybridized on 32k BAC arrays. Through cluster analysis two distinctive clusters related to genetic complexity were identified. One cluster consisted of tumors with complex genetic profiles with multiple gains and losses with an average of 41% of the genome altered, whereas the tumors of the other cluster had stable genetic profiles with an average of 18% of the genome altered. BAC clones are ordered according to their genomic position. Cytogenetic bands of regions of interest are labeled on the right.

HNPCC subset. DNA was extracted as described under '*DNA extraction, labeling and hybridization* 'and MSI status was determined using the markers BAT25, BAT26, BAT34C4, BAT40, D2S123, D5S346, D17S787 and a microsatellite in the *MSH6* gene. Primer sequences and analysis conditions are available from the authors upon request. The PCR products were analyzed on the ABI PRISM™ 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). MSI was defined as the presence of additional peaks and was classified as MSI low if only one marker showed instability and as MSI high if at least two markers showed instability.

Results

DNA copy number alterations. Unsupervised hierarchical cluster analysis split the 44 ovarian cancers into two major clusters related to the degree of genetic complexity without relation to histology, age or tumor status (Fig. 1). Complex alterations with mean 41% of the genome altered were found in 21/24 sporadic and 11/12 BRCA1 tumors, whereas stable genetic profiles, with mean 18% of the genome altered were identified in all 8 HNPCC tumors, in three of 24 sporadic tumors and in one of 12 BRCA1 tumors. The cluster analysis identified four outliers: three mucinous sporadic tumors and one serous BRCA1 tumor, which clustered among the tumors with stable genetic profiles. Gains on chromosomes 17 and 19 were commonly found in the genetically stable HNPCC-associated tumors and were present in 10 and 11 of the 12 tumors, respectively. MSI performed in the HNPCC associated tumors revealed MSI in 7/8 tumors with an MSI low pattern in five, and an MSI high phenotype in two.

Recurrent gains identified in at least 50% of BRCA1 and sporadic tumors included chromosomal regions 1q, 5p, 8q, 12p, 19p and 20 and losses on 4, 5q, 8p, 9q, 14q and 17p

Table II. Regions altered in ≥50% of the tumors.

Cytogenetic location	Start position (Mb)	Size (Mb)	% of tumors with change		P-value[a]	Candidate target genes
			Sporadic	HBOC	p<0.05	
Copy number gains ≥50%						
1q21.2-q25.1	146.8	23.8	53	23		*MUC1, UBE2Q1, RAB13, CREB3L4, CREG1*
5p15.33	0.000001	156.6	69	62		
6p22.1-p21.1	29.8	14.8	50	57		*TUBB, MD1L3, VEGF*
7p22.3	0.5	2	60	25		
7q36.3	156.1	2.2	21	50		
8q24.12-q24.3	119.9	26.4	51	72		*MYC*
10q26.3	134	1.2	52	42		
11q13.2-q24.3	65.8	6.6	34	50		*CCND1, FGF4, FGF3, EMSY*
12p13.33-p13.31	0.000001	8	68	60		*CD4 , KRAS*
16p13.3	0.5	2.6	60	33		
17p13.3	0.7	0.4	20	50		
19p13.11	17.6	1.6	54	43		*JAK3, JUND, RAB3A, ELL, MECT1, GDF1*
20p13	0.000001	3.1	74	51		*TRIB3, CSNK2A1, ANGPT4, CDC25B, GNRH2, ADAM33*
20q11.21-q13.33	29.4	33.1	73	43		
20q11.21-q11.22	29.7	3.4	87	50	**0.04**	*BCL2L1, PDRG1, RP11-49G10,8, E2F1, PAK1, TGIF2, RBL1, GHRH, SRC, TGM2*
20q13.12	45	5	66	58		
20q13.33	59.7	2.8	87	88		
Copy number losses ≥50%						
4p16-p14	10	27.3	29	50		
4q12-q31.21	53.2	93.6	46	63		*RASSF6, AREG, MAPK10, SCYE1, CASP6, PRDM5, GAB1, SMAD1*
4q22.1-q28.3	89.7	42	54	72		
4q31.23-q35.2	148.9	42.2	47	69		
4q34.3-q35.1	182.2	1.8	52	83	**0.005**	*VEGFC,CASP3*
5q11.2-q23.1	53.8	63.5	41	74		*ERBB2IP, ENC1, MSH3, EDIL3, RASA1*
7p21.3-p21.1	8.1	11.4	21	55		
8p23.3-p21.3	0.2	21.2	47	95	**0.002**	*TUSC3, MTUS1, NAT1, NAT2*
8p21.3-p12	23.3	13.8	42	77	**0.02**	*NRG1*
9p13.1-q21.11	38.8	30	36	53		
9q21.31-q21.32	79.2	3.8	52	42		*TBC1D2, TGFBR1*
9q22.33-q31.1	99.5	1	36	50		
9q33.1-q33.2	115.8	4.4	52	24		
12q14.1	56.9	3.4	4	50	**0.01**	
12q21.2-q23.1	76.9	23	15	56	**0.02**	
13q12.13-q32.2	25.1	72.1	35	72	**0.03**	*CDX2, FLT3, HSPH1, BRCA2, STARD13, LHFP, FOXO1A, LCP1, RB1*
14q12-q21.3	11	1.4	28	50		
14q22.1-q23.2	31.3	17.8	25	50		
14q24.3-q32.11	50.2	11.9	29	54		
14q32.33	77.7	10.6	38	50		
14q32.33	105.8	0.4	24	50		
15q.11.2	18.3	4.7	32	57		
16q21-q22.1	57	7.8	47	65		
16q22.1-q24.2	65.5	21	38	60		*TRADD, MMP2,E2F, ADAMTS18*
17p13.1-q21.2	8.7	28.1	30	65		*MAX,OVCA2, HIC1*

Table II. Continued.

Cytogenetic location	Start position (Mb)	Size (Mb)	% of tumors with change		P-value[a]	Candidate target genes
			Sporadic	HBOC	p<0.05	
17p12	10.9	4	67	73		
17p11.2	21.1	0.5	55	67		
17q22.1-q23.2	40.9	14.2	25	59	**0.03**	
18p11.32-p11.21	0.2	11.6	27	51		*BCL2, MALT1, SMAD4, SMAD2, SMAD7, SS18, DCC, CDH7*
18p11.21	14.1	0.9	11	61	**0.003**	
18q11.2-q23	17.3	58.9	53	68		
18q12.3	37.1	0.6	52	75		
18q21.1-q23	45.8	30	69	74		*BIRC7, CTSZ*
19p13.3	5	0.1	8	50	**0.0003**	
19p13.2	8.7	0.2	12	50	**0.04**	
21p13-q21.1	0.000001	22.4	34	52		*MYOB18B*
22q13.31-q13.33	4.3	56	37	51		*GTSE1*

[a]Determined using student's t-test.

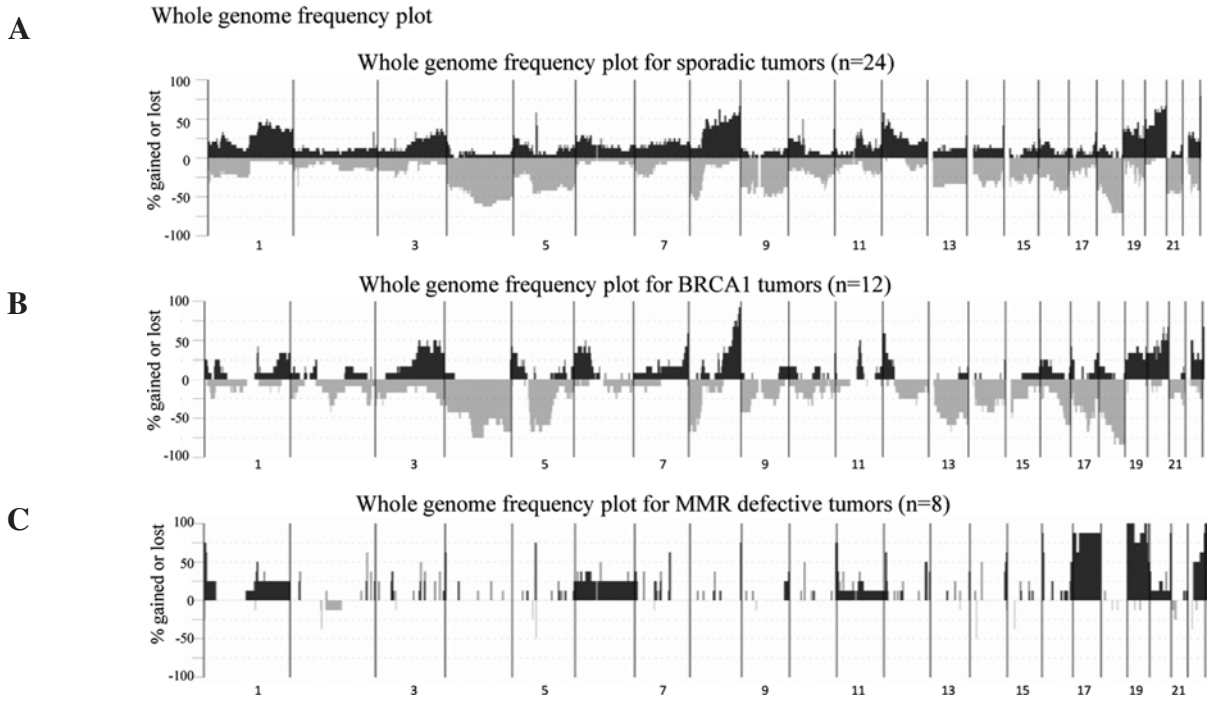

Figure 2. The frequency plot summarizes gains and losses in ovarian cancers from (A) 24 sporadic tumors, (B) 12 BRCA1 associated tumors and (C) 8 MMR defective tumors.

(Table II). Gains were more predominant in the sporadic tumors, whereas losses were more common in the BRCA1 tumors. When gains and losses present in ≥50% of the tumors were considered, losses of 4q34, 8p23-p21, 8p21-p12, 12q14.1, 12q21-q23, 13q12-q32, 17q22.1-q23.2, 18p11-p21, 19p13.3 and 19p13.2 were significantly over-represented in the BRCA1 tumors (p<0.05) (Table II). Potential discriminating genes within these regions include *BRCA2*, *FOXO1A* and *RB1*. Gains of 20q11.21-q11.22 were significantly more common in the sporadic tumors and harbor genes such as *SRC*, *E2F1* and *PAK1*. *BRCA1* promoter methylation pyrosequencing demonstrated promoter methylation in two tumors, both of which clustered in the genetically complex group (Fig. 1) (data not shown).

Table III. High level amplifications and homozygous deletions of genetically stable and complex ovarian tumors.

Tumor subset	Cytogenetic location	Start position (Mb)	Size (Mb)	Candidate target genes	Genetic profile	Histology
High level amplifications						
Sporadic	1p34.3-p34.2	36.4	7.0		Complex	Endometrioid
Sporadic	1p32.3	54.9	0.2		Complex	Serous
Sporadic	2p14-p13.3	67.9	1.1	*APLF, PROKR1*	Complex	Serous
Sporadic	2p11.2	84.4	1.0	*TMSB10, TCF7L1*	Complex	Serous
Sporadic	3p12.2-p12.1	81.7	2.3		Complex	Endometrioid
HBOC	3q26.1-q26.31	168.7	7.0	*TERC, TNFSF10, EIF5A2*	Complex	Serous. endometrioid
Sporadic	3q27.1-p27.2	185.3	0.8		Complex	Serous
Sporadic	4p16.3	0.01	2.7	*FGFRL1, CTBP1, TACC3*, *FGFR3, WHSC1, WHSC2*	Complex	Serous
Sporadic	4q13.3	73.9	1.1	*AFP, IL-8*	Complex	Serous
HBOC	5p15.33-p15.1	0.1	18.3	*TERT, ADAMTS16*	Complex	Serous
Sporadic	5p13.3-p13.2	33.0	4.5	RAD1, SKP2	Complex	Serous
Sporadic	6p22.3	18.7	4.8	*E2F3*	Complex	Serous
HBOC	6p21.32-p21.2	33.4	3.8	*HMGA1, PPARD*, *CDKN1A, PIM1*	Complex	Endometrioid
Sporadic	6p21.2-p21.1	37.6	6.2	*CCND3*	Complex	Serous
Sporadic	8p21.2-p21.1	26.7	1.4		Complex	Serous
HBOC. sporadic	8p12-p11.23	36.7	1.9	*FGFR1*	Complex	Serous
HBOC	8p11.21	41.8	0.9	*IKBKB, POLB*	Complex	Serous
HBOC. sporadic	8q24.21	127.8	3.2	*MYC, PVT1*	Complex	Serous
Sporadic	10p11.22	32.5	1.6	*NRP1*	Complex	Serous
Sporadic	10q21.1-q21.3	59.9	5.2		Complex	Serous
HBOC. sporadic	11q13.4-q13.5	74.0	1.2	*EMSY*	Complex	Serous
Sporadic	11q14.1	77.7	2.6		Complex	Serous
Sporadic	11q22.1-q22.2	99.4	2.4	*PGR*	Complex	Serous
Sporadic	11q22.2	101.8	0.5	*Matrix metalloproteinase (MMP) family cluster*	Complex	Serous
Sporadic	13q22.3-q31.1	76.9	1.2		Complex	Serous
HBOC	14q13.1-q13.3	33.8	2.8		Complex	Endometrioid
Sporadic	15q22.2	58.4	1.7	*ANXA2*	Complex	Serous
Sporadic	16p11.2	29.5	2.0		Complex	Serous
Sporadic	17p13.1	7.0	2.1	*TP53*	Complex	Serous
Sporadic	17q12-q21.1	35.0	0.8	*ERBB2*	Complex. stable	Serous. mucinous
Sporadic	18q11.2	18.0	2.1	*RBBP8, cables 1*	Complex	Serous
Sporadic	18q21.1	44.0	0.9		Stable	Mucinous
Sporadic	18q21.33	58.1	0.7	*BCL2*	Stable	Mucinous
Sporadic	18q22	73.3	0.4		Stable	Mucinous
Sporadic	19p13.13-p13.12	13.7	0.7		Complex	Serous. endometrioid
Sporadic	19q12	33.7	1.4	*CCNE1*	Complex	Serous
Sporadic	19q13.32	50.6	1.1	*FOSB, HIF3A*	Complex	Serous
HBOC	20p13	0.03	4.6	*CDC25B*	Complex	Endometrioid
HBOC. sporadic	20q13.12-q13.13	43.1	3.4	*WFDC2, MMP9*,	Complex	Serous
Sporadic	20q13.13	47.1	2.0	*CSE1L, PTPN1, ADNP*	Complex	Serous
HBOC	20q13.33	60.0	0.3	*CDH4, BIRC7*	Complex	Endometrioid
Sporadic	22q12.3	30.5	1.6		Complex	Serous

Table III. Continued.

Tumor subset	Cytogenetic location	Start position (Mb)	Size (Mb)	Candidate target genes	Genetic profile	Histology
Homozygous deletions						
HBOC	4q35.2	187.9	0.2	*FAT1*	Complex	Serous
HBOC	5q12.3-q13.1	66.3	1.4		Complex	Serous
HBOC	9p21.3-p21.1	21.5	0.6	*CDKN2A, CDKN2B*	Complex	Serous
HBOC	10q23.31	90.0	0.4	*PTEN*	Complex	Endometrioid
Sporadic	14q31.3	83.7	2.2		Complex	Serous

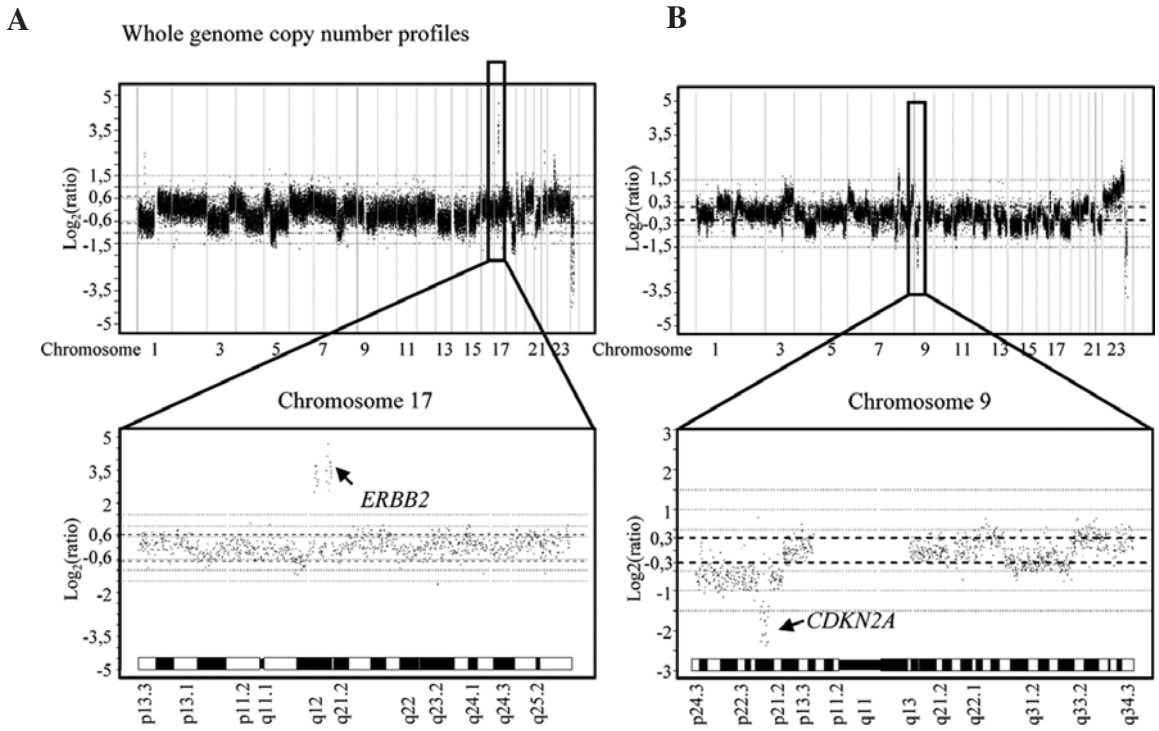

Figure 3. Pictured are two ovarian cancers demonstrating multiple gains and losses. (A) Characteristic alterations for sporadic tumors include gains on 1q, 5p and 20q and losses on 4q and 18q. Zoom-in on chromosome 17 reveals a high level amplification on 17q12, harboring the ERBB2 gene. (B) Characteristic alterations for BRCA1 tumors include gains on 6p and 8q and losses on 4q, 5q and 8p. Zoom-in on chromosome 9 reveals a probable homozygous deletion on 9p21.3, harboring the CDKN2A gene.

High level amplifications and homozygous deletions. High level amplifications (segments with $\log_2$ ratios >5 times the adaptive threshold) that affected 42 different loci were observed in 18 tumors. High level amplifications were as common in the sporadic tumors (12/24) as in the BRCA1 tumors (7/12) and were not observed in any HNPCC associated tumors (Table III). Recurrent high level amplification involved the *ERBB2* locus on 17q12 in two sporadic tumors, *EMSY* (11q13.5) in three tumors (one BRCA1 and two sporadic) and *MYC* (8q24.21) in one BRCA1 and one sporadic tumor. Six tumors (two sporadic and four BRCA1 tumors) showed putative homozygous deletions (segments with $\log_2$ ratios <7 times the threshold) in 9 loci including 4q35 (*FAT*), 9p21.3-p21.1 (*CDKN2A*, *CDKN2B*) in two tumors and 10q23.31 (*PTEN*) (Table III, Fig. 3). Significantly altered regions identified by STAC are listed in Table IV.

Discussion

The multitude of copy number changes demonstrated in the sporadic tumor subset included several alterations previously linked to ovarian cancer, e.g. gains at 1q, 3q (*PIK3CA*), 8q (*MYC*), 11q (*PAK1, CCND1, FGF4/FGF3*), 12p (*KRAS2*), 17q (*ERBB2*), 20q (*STK15*) and recurrent losses of 4q, 9p (*CDKN2A*), 13q (*BRCA2*) and 16q (Table II) (21-23).

Table IV. Significant testing for aberrant copy number analysis.

Tumor subset	Cytogenetic location	Start position (Mb)	Size (Mb)	Candidate target genes
Copy number gains				
HBOC	1q21.3-q22	151.1	2.2	*MUC1, UBE2Q1, RAB13, CREB3L4, CREG1*
HBOC	5q35.2-q35.3	175.1	5.7	*NSD1, MAPK9, GNB2L1*
HBOC	8q24.22	132.8	3	
HBOC	8q24.3	140.5	5.5	*PSCA, MAFA, BOP1, RECQL4*
HBOC	9q31.3-q34.3	111.7	26.3	*TNC, DAB2IP, SET, PPP2R4, PRRX2, FNBP1, ABL1, NUP214, TSC1, NOTCH1*
HBOC	11q13.2-q13.4	65.6	5.3	*CCND1, FGF4, FGF3*
HBOC	12p13.33-p13.31	0.000001	6.9	*CCND2*
HBOC	17p13.3-p13.1	0.00001	7.9	*TP53*
HBOC	17q24.3-q25.3	67.9	10.8	*GRB2, RECQL5, ST6GALNAC1, SEPT9, ENPP7, ASPSCR1, RAC3*
HBOC	20q13.33	61.2	1.2	
HBOC	22q11.21-q11.22	16.3	4.6	*CLTCL1, sept52L1, PPIL2,*
HNPCC	2p23.3	27.3	0.4	
HNPCC	2q35	219.9	0.4	
HNPCC	3p21.31	49.5	0.9	*MST1R, RBM5, SEMA3F, HYAL1, HYAL2, RASSF1*
HNPCC	3q21.3	130.2	0.4	
HNPCC	4p16.3	1.0	1.9	*CTBP1, TACC3, FGFR3, WHSC1, WSC2*
HNPCC	6q13	74.0	0.5	*DDX43*
HNPCC	7q22.1	99.5	1.5	
HNPCC	11p15.5-p15.4	0.000001	3.9	*HRAS, CARS, NUP98*
HNPCC	12p13.31	6.2	1.9	*ZNF384*
Sporadic	2q37.1	232.2	1	
Sporadic	3p21.31	50.4	0.3	
Sporadic	4p16.3-p16.1	0.1	8.4	*FGRGL1, CTBP1, TACC3, FGFR3, WHSC1, WHSC2*
Sporadic	5q13.2	68.5	1	*CCNB1*
Sporadic	8p11.22-p11.1	39.8	5.3	*IKBKB, POLB*
Sporadic	9q34.11-q34.3	127.7	10.3	*SET, PPP2R4, PRRX2, FNBP1, ABL1, NUP214, TCS1, NOTCH1*
Sporadic	10p11.22	31.8	1	
Sporadic	10q11.21	42.9	1.7	*RET*
Sporadic	11q13.5	75.8	0.8	*EMSY*
Sporadic	12p13.33-p13.31	0.000001	6.9	*CCND2*
Sporadic	13q14.11	43.5	0.5	
Sporadic	13q14.2	49.1	0.5	
Sporadic	16q11.2	40.7	4.4	
Sporadic	16q23.1	76.1	1	
Sporadic	17q25.3	76.7	2	*ASPSCR1, RAC3*
Sporadic	20q13.33	61.3	0.8	
Sporadic	21q22.3, end	46.6	0.3	*S100B*
Copy number losses				
HBOC	3q27.2-q29	187.2	7.3	*ETV5, EIF4A2, BCL6, LPP*
HBOC	5q11.2-q13.2	55.1	13.3	*ERBB2IP, ENC1*
HBOC	8p23.3-p21.3	0.5	19.6	*TUSC3, MTUS1, NAT1, NAT2, GATA4*
HBOC	9p24.3-p21.1	0.4	27.7	*JAK2, JMJD2C, PTPRD, PSIP1, SH3GL2, MLLT3, CDKN2A, CDKN2B*
HBOC	9q22.32-q31.1	95.8	4.8	
HBOC	16q23.1-q24.3	74.4	14.4	*FANCA*

Table IV. Continued.

Tumor subset	Cytogenetic location	Start position (Mb)	Size (Mb)	Candidate target genes
Copy number losses				
HBOC	19p13.2	8.7	1	
Sporadic	1p36.32-p36.31	4.0	1.6	
Sporadic	5q11.2-q13.2	51.7	16.7	*ERBB2IP, ENC1*
Sporadic	8p23.2	2.7	0.1	
Sporadic	8p23.1-p21.3	9.9	10.2	
Sporadic	10q25.1	106.3	1.3	
Sporadic	13q12.11-q14.2	18.4	30.7	*CDX2, FLT3, HSPH1, BRCA2, STARD13, LHFP, FOXO1A, LCP1, RB1*
Sporadic	14q32.33	105.3	1	
Sporadic	17q22	49.0	1.4	

Data on BRCA1 ovarian cancer profiles suggest genetic complexity with gains of 2q, 3q, 6p, 7q, 8q, 20q, and losses of 4q, 5q, 6q, 13q, 17q, 18q, 19, 20q, which is supported also by our findings (24,25). Similarities have also been recognized between HBOC associated and sporadic breast cancer, e.g. with losses of 4q and 5q in both tumor types and BRCA1 inactivation is thus linked to genetically complex tumors without yet identified differences from the sporadic ovarian cancers (26-29). All eight HNPCC associated tumors were genetically stable. Recurrent gains affected chromosomes 17 and 19, though these regions are too large to allow distinction of potential target genes (Table II). The stable genetic profiles demonstrated in the HNPCC associated ovarian cancers are in line with the findings in colorectal cancer, in which tumors with germline MMR gene mutations or *MLH1* promotor methylation display near diploid genetic profiles with few structural changes (30-32).

STAC analysis was applied to the different tumor subsets in order to identify target genes within the regions specifically gained or lost (Table IV). A region on 17p13 harboring the *TP53* gene was gained in a significant number of BRCA1 tumors. While point mutations is the most common mechanism for TP53 inactivation in ovarian cancer, amplifications has been reported in endometrioid cancers, which underscores that different genetic mechanisms apply in the development and progression of the various types of ovarian cancer (2,33). Further, a number of oncogenes commonly gained in ovarian cancer such as *HRAS* and *EMSY* were identified. Mutations of *EMSY* are associated with poor outcome and serous histology. Candidate genes involved in tumor suppression included *BRCA2* in the sporadic subset, *GATA4* and *FANCA* in the BRCA1 tumors (34). Loss of FANCA proteins implicates that inhibition of the DNA damage signalling poly (ADP-ribose) polymerase (PARP) protein may be useful in a subset of sporadic tumors (35).

High level amplifications were predominantly found in sporadic and *BRCA1* ovarian cancers and included the potential target genes *PGR*, *CDCN20*, *SMAD7*, *TIMP3*, *PAK1* and *ERBB2* (Table III, Fig. 3). The *ERBB2* locus at 17q12 plays a critical role in tumor cell proliferation with overexpression in up to three quarters of ovarian cancer (36). Frequent high level amplifications occurred in both sporadic and BRCA1 mutated tumors, which suggests that copy number dependent oncogene amplifications are central in these tumor subsets. Two BRCA1 tumors showed homozygous deletions of the *CDKN2A* locus, which has been observed in 11-27% of ovarian cancers, and has been linked to advanced stage and poor prognosis (Table III, Fig. 3) (37,38). Lower degree of oncogene activation in MMR defective ovarian cancers compared to the *BRCA1* mutated and sporadic cases may have therapeutic consequences for the response to chemotherapeutic regimens and targeted therapies. *BRCA1* mutated ovarian cancers are generally resistant to paclitaxel, but responsive to platinum based drugs, whereas *in vitro* data suggest that MMR defective cells may be resistant to platinum based chemotherapy (39-41). Inhibition of *ERBB2* and *MYC* has been suggested to be less effective in hereditary ovarian cancers, whereas PARP inhibition and anti-angiogenic agents show promising results in HBOC associated ovarian cancers (35,36,42).

The distinct genetic profiles recognized in the two major types of hereditary ovarian cancer reflect tumor development along different pathways with genetic complexity in the HBOC associated *BRCA1* mutated tumors and stable genetic profiles in the HNPCC associated MMR defective tumors. This is in concordance with the predominance of serous tumors in HBOC and endometrioid, clear cell, and mucinous tumors commonly diagnosed in HNPCC. A dualistic model for the development of ovarian cancer has been suggested with high grade serous and undifferentiated tumors developing *de novo*, whereas low grade serous carcinomas, mucinous, clear cell and low grade endometrioid tumors develop in a stepwise sequence (43). Cellular origin may differ with HBOC associated ovarian cancers suggested to originate in the fallopian tube secretory epithelium rather than the surface epithelium (44,45). Different genetic pathways drive tumor development with mutations in *TP53*, *PI3K/AKT* pathway genes and *BRCA1/BRCA2* in the genetically

unstable cases and mutations in *KRAS, BRAF, PTEN* and *ß-catenin* frequently identified in the genetically stable ovarian cancers (2,43,46,47). The differences in genetic complexity identified may be applied for refined diagnostics of HBOC and HNPCC associated ovarian cancer, and may also have implications for the response to chemotherapy and targeted therapies for individuals with hereditary ovarian cancer.

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