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Molecular genetic evidence that endometriosis is a precursor of ovarian cancer

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Histopathology and epidemiology studies have consistently demonstrated a strong link between endometriosis and endometriosis-associated ovarian cancers (EAOCs)—in particular, the endometrioid and clear cell subtypes. However, it is still unclear whether endometriosis is a precursor to EAOCs, or whether there is an indirect link because similar factors predispose to both diseases. In order to search for evidence of clonal progression, we analyzed 10 EAOCs (endometrioid = 4; clear cell = 6) with coexisting endometriosis for common molecular genetic alterations in both the carcinoma and corresponding endometriosis. We used 82 microsatellite markers spanning the genome to examine loss of heterozygosity (LOH) in the coexisting carcinoma and endometriosis samples. A total of 63 LOH events were detected in the carcinoma samples; twenty two of these were also detected in the corresponding endometriosis samples. In each case, the same allele was lost in the endometriosis and cancer samples. Interestingly, no marker showed LOH in the endometriosis alone. These data provide evidence that endometriosis is a precursor to EAOCs.
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Key words: endometrioid ovarian cancer; clear cell ovarian cancer; endometriosis; precursor lesions; loss of heterozygosity

Ovarian cancer ranks fourth in cancer deaths among women aged 45–64 years, and is the most lethal of all gynecological cancers (http://seer.cancer.gov/). The high mortality rate arises mainly because the disease is asymptomatic in its initial stages, making its early detection difficult.[1] At the time of diagnosis, dissemination has occurred in more than 70% of cases, at which point the 5-year survival rate is less than 20%. Ninety percent of all ovarian cancers are epithelial in origin, and are classified according to their cell types (serous, mucinous, endometrioid, clear cell and undifferentiated or mixed histology).[1] Different etiological factors have been implicated in these subtypes[2] although, at present, little is known about the molecular events involved in their individual development.

Endometrioid and clear cell subtypes have been associated with the benign disease, endometriosis. This is a complex genetic trait which affects up to 10% of women in their reproductive years.[3] It causes pelvic pain, severe dysmenorrhea (painful periods) and subfertility.[4] The disease is defined by the presence of endometrial-like epithelium and stroma in the extra-uterine sites, most commonly the ovaries and peritoneum. The main pathological processes associated with endometriosis are peritoneal inflammation and fibrosis, and the formation of adhesions and endometriomas (benign ovarian cysts).

Circumstantial evidence that endometriosis is an endometriosis-associated ovarian cancer (EAOC) precursor has been accumulating over many years. Frequent coexistence of endometrioid and clear cell ovarian cancers with endometriosis has been reported in several pathology case series.[5–13] In approximately 60% of EAOCs, the cancer is adjacent to, or arises directly from, endometriosis tissue, suggesting that malignant transformation occurs.[6,8] The age-adjusted incidence rate of ovarian cancer is 13.9 per 100,000 women (0.0139%) (http://seer.cancer.gov/), with endometrioid and clear cell carcinomas accounting for only 14 and 6% of all ovarian carcinomas, respectively. However, it has been reported that 5–10% of women with ovarian endometriomas have ovarian carcinomas,[6,14] with clear cell and endometrioid carcinomas by far the predominant EAOCs (40–55% clear cell, 20–40% endometrioid and <10% serous/mucinous).[5,8,13] Although many of the risk factors associated with both diseases are similar, including earlier menarche, more regular periods, shorter cycle length and lower parity, endometriosis itself may be a risk factor for ovarian cancer. After adjusting for age, number of pregnancies, family history of ovarian cancer, race, oral contraceptive use, tubal ligation, hysterectomy and breastfeeding; women with ovarian cancer were 1.7-fold more likely to have reported a history of endometriosis than controls.[15] These data are consistent with an earlier report of a 2.5-fold increased risk of ovarian cancer in endometriosis patients, with the risk increasing to 4.2-fold in those with a history of ovarian endometriosis.[16]

However, despite the histological and epidemiological evidence linking these two diseases, it is still unclear whether endometriosis is a precursor to EAOCs, or whether there is an indirect link involving common environmental, immunological, hormonal or genetic factors.[17] Molecular genetic studies of allele loss have been used to demonstrate clonal progression from precursor lesion to cancer.[18–20] In order to search for evidence of clonal progression from endometriosis to cancer, we analyzed 10 EAOC cases with coexisting endometriosis for common molecular genetic alterations in both the tumor and corresponding endometriosis, using 82 microsatellite markers spanning the genome.

Material and methods

Tissue samples and microdissection

Unlinked, anonymised, archival specimens from 10 women, with ovarian endometriosis and cancer (Table I; Fig. 1), were obtained from the John Radcliffe Hospital, Oxford, UK; Birmingham Women's Hospital, Birmingham, UK; Fox Chase Cancer Center, Philadelphia, PA; and M.D. Anderson, Houston, TX. IRB approval to collect and evaluate tissue samples had been obtained from each participating institute. Histological sections were prepared from formalin-fixed, paraffin-embedded tissue. Sections were cut from blocks representing each lesion; one section was stained with hematoxylin and eosin (H&E) for review by a histopathologist, and 5 sections were lightly stained with hematoxylin

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TABLE I – CLINICAL FEATURES OF THE 10 SAMPLES USED IN THE ANALYSIS, INCLUDING AGE OF DIAGNOSIS, TUMOR HISTOLOGY, STAGE AND SITE OF THE OVARIAN ENDOMETRIOSIS IN RELATION TO ADENOCARCINOMA

Sample	Age	Carcinoma	Stage	Site of endometriosis
146	66	Endometrioid	1A	Adjacent
2165	54	Endometrioid	1B	Adjacent
914	48	Endometrioid	1B	Contralateral
247	41	Clear cell	1A	Adjacent
18542	53	Mixed, predominantly endometrioid with some clear cell foci[1]	–	Adjacent
11M	60	Clear cell	1C	Adjacent
10M	32	Clear cell	Borderline	Adjacent
3870	77	Clear cell	1B	Contralateral
8872	61	Mixed, predominantly clear cell with some endometrioid foci[1]	1A	Contralateral
9684	48	Clear cell	1C	Contralateral

[1]The predominant carcinoma was analyzed.

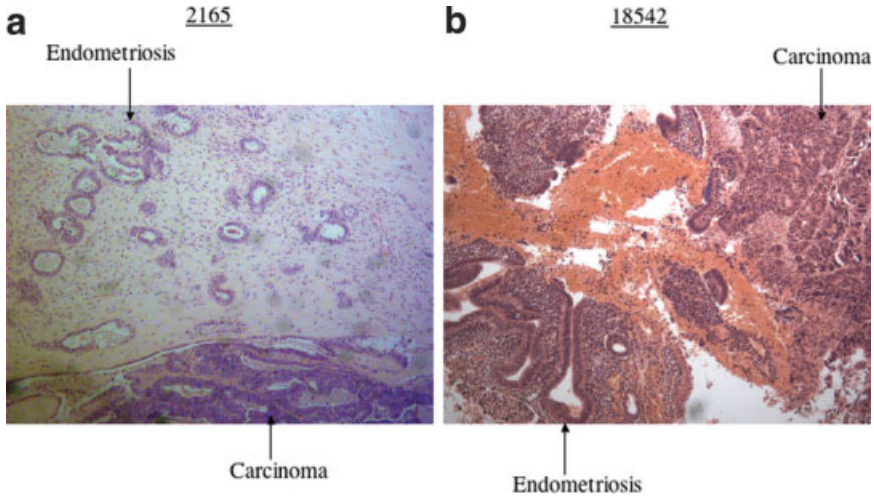

FIGURE 1 – (*a*) Histology of case 2165: H&E (×10), showing adjacent endometriosis and endometrioid ovarian cancer. (*b*) Histology of case 18542: H&E (×10), adjacent endometriosis and endometrioid ovarian cancer.

for microdissection. Each section was microdissected using either the Leica AS LMD system (Leica Microsystems, Wetzlar, Germany) or the PixCell II Laser Capture Microdissection system (Arcturus Engineering, Mountain View, CA), to separate the endometriosis, the carcinoma and surrounding normal stromal tissue. DNA extraction was performed using the Qiagen QIAamp DNA Micro kit, according to the manufacturer's instructions (Qiagen, Crawley, Sussex, UK).

Whole genome amplification

Whole genome amplification (WGA) was performed to gain sufficient DNA for loss of heterozygosity (LOH) analysis, because only minute quantities of DNA could generally be obtained from the microdissected endometriosis and carcinoma samples. DNA was amplified using the GenomePlex WGA kit (Sigma-Aldrich, Dorset, UK), according to the manufacturer's instructions. The GenomePlex WGA kit is based on random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR-amplifiable library molecules flanked by universal priming sites. WGA is achieved by PCR amplification of the library molecules using universal oligonucleotide primers. It has been used successfully by other groups, and has shown no detectable locus or allele bias.[21,22] We found that this method worked well for amplifying DNA from archival formalin-fixed, paraffin-embedded samples. Whole genome amplified DNA was purified using the Qiagen MinElute PCR purification kit (Qiagen), prior to LOH analysis.

LOH analysis

Polymorphic microsatellite markers were chosen from all 22 autosomal chromosomes (The Wellcome Trust Centre for Human Genetics, Oxford or Applied Biosystems, Cheshire, UK). Owing to the poor quality of the DNA from our archival samples, the markers were chosen based on the small size (<200 bp) of the product. The following microsatellite markers were used: D1S230; D1S498; D1S213; D2S319; D2S162; D2S165; D2S391; D2S160; D2S112; D2S2330; D2S396; D2S125; D3S1286; D3S1547; D3S1271; D3S1614; D3S1311; D4S2935; D4S392; D4S407; D4S1586; D5S648; D5S407; D5S433; D6S470; D6S1568; D6S1627; D6S262; D6S1577; D7S2532; D7S484; D7S494; D7S515; D8S264; D8S261; D8S270; D8S272; D9S285; D9S1877; D9S1776; D9S164; D10S548; D10S196; D10S537; D10S215; AFMa086wg9; D10S587; D10S212; D11S4046; D11S4191; D11S1314; D11S1793; D12S83; D12S79; D12S1723; D13S175; D13S153; D13S265; D13S158; D14S283; D14S275; D14S70; D14S63; D14S65; D15S1002; D15S987; D16S3075; D16S520; D17S799; D17S787; D17S785; D18S63; D18S452; D18S1102; D19S884; D19S221; D19S210; D20S178; D20S171; D21S1256; D21S266; D22S83 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists; http://www.genlink.wustl.edu/genethon_frame/): One oligonucleotide from each pair was fluorescently labeled with FAM, HEX or NED (The Wellcome Trust Centre or Applied Biosystems). PCR was performed in a 20 µl reaction mixture containing 1× Qiagen PCR Master Mix (Qiagen), 0.5 µM concentrations of each primer pair and 30 ng of DNA from the microdissected endometriosis, ovarian carcinoma or normal ovarian stromal tissue. PCR amplification was performed as follows: 95°C for 5 min, 35 cycles of 96°C for 1 min, an annealing step of 53°C for 1 min, 72°C for 1 min, followed by a final extension step of 72°C for 10 min in an Eppendorf PCR machine. The PCR products were run on an ABI 3700 Genetic Analyzer, and microsatellites were analyzed for allelic imbalance using the Genotyper program (Applied Biosystems).

TABLE II – SUMMARY OF LOH RESULTS, SHOWING THE NUMBER OF LOH EVENTS FOR EACH SAMPLE, TOGETHER WITH THE NUMBER OF MARKERS THAT WERE INFORMATIVE IN THE ANALYSIS

Sample	Clinical details	LOH events/informative markers	Microsatellite marker and loci at which LOH was detected
146T	Endometrioid	1/19	D18S452 (18q12.2)
146E	Adjacent	0/39	–
2165T	Endometrioid	3/51	D2S319 (2p25); D7S515 (7q22); D8S272 (8q24)
2165E	Adjacent	1/28	D7S515 (7q22)
914T	Endometrioid	4/28	D5S407 (5q11.2); D9S285 (9p23-22); D13S175 (13q11); D16S520 (16q24)
914E	Contralateral	3/10	D5S407 (5q11.2); D13S175 (13q11); D16S520 (16q24)
247T	Clear cell	11/39	D3S1311 (3q26-27); D5S658 (5p14); D5S433 (5q21); D6S1568 (6p21); D7S494 (7p13-11.1); D9S285 (9p23-22); D10S548 (10p14-12); D10S215 (10q23); D13S265 (13q31); D14S283 (14q11); D14S275 (14q12)
247E	Adjacent	2/45	D5S433 (5q21); D13S265 (13q31)
18542T	Endometrioid	11/49	D5S407 (5q11.2); D5S433 (5q21); D6S470 (6p24.3); D6S1627 (6p14-15); D10S537 (10q23); D11S4046 (11p15.5); D11S4191 (11p11.2); D11S1314 (11q13.1); D13S265 (13q31); D17S787 (17q22-23.2); D18S452 (18p11)
18542E	Adjacent	4/51	D6S470 (6p24.3); D10S537 (10q23); D11S4191 (11p11.2); D17S787 (17q22-23.2)
11MT	Clear cell	9/44	D3S1614 (3q26.2); D3S1311 (3q26.32-3q27); D6S470 (6p24.3); D10S537 (10q22.1); D10S212 (10q26); D13S265 (13q31); D17S787 (17q22-23.2); D18S452 (18p11); D22S283 (22q12)
11ME	Adjacent	6/39	D3S1614 (3q26.2); D3S1311 (3q26.32-3q27); D6S470 (6p24.3); D10S537 (10q22.1); D13S265 (13q31); D17S787 (17q22-23.2)
10MT	Clear cell	4/43	D4S407 (4q24); D14S283 (14q11.1-q12); D19S884 (19p13); D21S1256 (21q22)
10ME	Adjacent	2/41	D4S407 (4q24); D21S1256 (21q22)
3870T	Clear cell	0/16	–
3870E	Contralateral	0/5	–
8872T	Clear cell	15/46	D2S319 (2p25); D2S2330 (2q12-13); D3S1311 (3q26.32-q27); D5S407 (5q11.2); D10S537 (10q23); D10S215 (10q23); D11S4046 (11p15.5); D11S1314 (11q13.1-q14.3); D12S79 (12q24.21); D13S265 (13q31.2-q31.3); D14S65 (14q32); D17S799 (17p12); D17S785 (17q25); D19S221 (19p13.2-13.3); D20S171 (20q13)
8872E	Contralateral	2/51	D11S1314 (11q13.1-q14.3); D14S65 (14q32)
842T	Clear cell	5/47	D3S1286 (3pter-3p24.2); D3S1311 (3q26.32-3q27); D5S507 (5q11.2); D12S1723 (12q13); D17S787 (17q22-23.2)
842E	Contralateral	2/40	D5S507 (5q11.2); D17S787 (17q22-23.2)
All T		*63*	
All E		*22*	

Clinical details (histology of the ovarian tumor and the site of the endometriosis in relation to the tumor) and the loci of the markers which showed LOH are shown. Common LOH events have been identified in endometriosis and in both endometrioid and clear cell ovarian tumors and in samples which are in the same and in contralateral ovaries.

In informative (heterozygous) cases, allelic loss at each marker was scored in the endometriotic lesion or carcinoma if the area under one allelic peak was reduced by >50% relative to the other allele, after correcting for the relative peak areas in DNA from the normal tissue.

Results

In order to search for evidence of clonal progression, we analyzed 10 EAOC cases with coexisting endometriosis (Fig. 1), for common molecular genetic alterations. LOH analysis used 82 microsatellite markers. At least one marker from all the autosomal chromosome arms, except 12p and 20p, were used in the analysis. Thirty-seven of the 82 markers showed LOH in at least 1 carcinoma sample. A total of 63 LOH events were detected in the carcinoma samples, and 22 of these LOH events were also detected in the corresponding endometriosis (Tables II and III; Fig. 2*a*). No markers showed LOH in the endometriosis alone, and all 22 LOH events involved the same allele in both the carcinoma and endometriosis (Table III and Fig. 2*a*). LOH at one or more markers was detected in all but 1 carcinoma, and all but 2 endometriosis samples (Table II).

WGA, PCR amplification and LOH analysis were performed twice on samples where LOH was identified, and in every case, identical results were found both times (data not shown). In addition, using the original DNA (prior to WGA) from normal and carcinoma from sample 842, we were able to confirm LOH at D17S787 (data not shown).

In all 9 lesions in which LOH was detected, LOH was detected more frequently in the carcinoma than in the endometriosis (Table II; Fig. 2*b*). This suggests that the endometriosis samples were not contaminated with carcinoma, and is consistent with the fact that all our samples were obtained by laser microdissection. LOH in both the endometriosis and carcinoma was detected on chromosomes 3q, 4q, 5q, 6p, 7q, 10q, 11p 11q, 13q, 14q, 16q, 17q and 21q (Tables II and III). Additional LOH events were detected in the carcinomas alone on chromosomes 2p, 2q, 3p, 5p, 6q, 7p, 8q, 9p, 10p, 12q, 14p, 17p, 18p, 18q, 19p, 20q and 22q (Table II). D5S407 at 5q11.2, D6S470 at 6q24.3 and D13S265 at 13q31.2-q31.3 showed LOH in 2 endometriosis/tumor pairs and D17S787 at 17q22-23.2 showed LOH in 3 endometriosis/carcinoma samples (Table III).

Assuming equal probabilities of loss of paternal and maternal alleles, the probabilities of losing the same allele for each LOH event is $p = 0.5$. Therefore, the probability of identical alleles being lost by chance in each case, was $p = 0.5$ for sample 2165, where there was one common LOH event; $p = 0.5^2 = 0.25$ for samples 247, 10M, 8872 and 842, where there were 2 markers showing LOH of the same allele; $p = 0.5^3 = 0.125$ for sample 914, showing 3 LOH events in common; $p = 0.5^4 = 0.0625$ for 18542, showing 4 events in common; and $p = 0.5^6 = 0.015$ for sample 11M, where there were 6 LOH events in common (Table III). The likelihood of identical alleles being lost in both endome-

TABLE III – RESULTS FROM THE LOH ANALYSIS OF CHROMOSOMES

Marker	cM	2165T	2165E	914T	914E	247T	247E	18542T	18542E	11MT	11ME	10MT	10ME	8872T	8872E	842T	842E
Chrm 3q																	
D3S1614	169.9	H	–	H	–	H	H	H	H	L	L	–	–	H	H		
D3S1311	220.1	N	N	–	–	L	H	H	H	L	L	–	–	L	H	L	H
Chrm 4q																	
D4S407	101	H	–	–	–	–	H	H	H	–	–	L	L	–	H	H	H
D4S1586	146.6	–	–	N	N	N	N	N	N	–	–	N	N	–	–	H	H
Chrm 5q																	
D5S407	65	H	H	L	L	H	H	L	H	N	N	H	H	L	H	L	L
D5S433	112.3	H	–	N	N	L	L	L	H	H	H	–	H	–	–	H	–
Chrm 6p																	
D6S470	17.7	H	H	H	H	–	–	L	L	L	L	H	H	H	H	H	–
D6S1568	47.6	–	–	H	–	L	H	H	H	–	–	H	H	H	H	H	H
Chrm 7q																	
D7S515	112.9	L	L	–	–	–	–	H	H	H	H	–	–	–	H	–	–
Chrm 10q																	
D10S537	93.8	N	N	–	–	H	H	H	H	L	L	–	–	H	H	N	N
D10S215	113	H	–	–	–	H	H	L	L	–	–	H	–	L	–	H	–
AFMa086wg9	114	–	–	H	–	L	H	H	H	–	–	H	H	L	H	H	H
D10S587	156.6	H	–	–	–	N	N	H	H	H	H	–	–	–	–	–	–
D10S212	180.7	–	–	–	–	N	N	H	H	L	–	–	–	–	–	H	–
Chrm 11p																	
D11S4046	3.9	–	–	–	–	–	–	L	H	H	H	H	–	L	H	N	N
D11S4191	63.4	–	–	H	H	H	H	L	L	H	H	H	H	H	H	H	H
Chrm 11q																	
D11S1314	77.5	H	H	H	–	–	H	L	H	–	–	H	H	L	L	H	H
D11S1793	110.3	–	–	–	–	H	H	H	H	–	–	H	H	H	H	H	H
Chrm 13q																	
D13S175	7.4	–	–	L	L	–	–	N	N	N	N	–	H	–	–	N	N
D13S153	47.5	H	–	H	–	H	H	N	N	H	H	N	N	N	N	N	N
D13S265	70.6	H	–	H	–	L	L	L	H	L	L	H	H	L	H	H	H
D13S158	86.9	H	–	H	–	–	–	H	H	H	H	–	–	N	N	H	H
Chrm 14q																	
D14S283	7.5	H	–	–	–	L	H	N	N	H	H	L	–	H	H	–	–
D14S275	21.9	–	–	H	–	L	–	–	–	H	H	H	H	N	N	H	H
D14S70	32.9	H	–	H	–	H	H	H	H	H	–	H	H	H	H	H	H
D14S63	59	–	–	H	–	H	–	N	N	H	H	H	H	H	H	–	–
D14S65	108.1	H	–	–	–	–	H	H	–	–	H	H	–	L	L	–	–
Chrm 16q																	
D16S3075	21.8	H	–	H	H	H	–	N	N	–	–	H	H	–	–	–	–
D16S520	123.3	–	–	L	L	–	–	H	–	H	H	H	H	H	H	N	N
Chrm 17q																	
D17S787	75.7	–	–	–	–	–	H	L	L	L	L	H	H	N	N	L	L
D17S785	104.7	H	–	–	–	–	–	–	H	H	H	H	H	L	H	H	H
Chrm 21q																	
D21S1256	8.6	H	H	–	–	–	–	N	N	H	H	L	L	–	–	N	N
D21S266	49.9	H	–	–	–	–	–	–	H	–	–	N	N	H	H	H	H

LOH analysis of chromosomes 3q, 4q, 5q, 6p, 7q, 10q, 11p, 11q, 13q, 14q, 16q, 17q and 21q shows 8 cases of ovarian endometriosis and corresponding ovarian tumor, where LOH of the same marker (and same allele) was detected in both the endometriosis and corresponding carcinoma. In one case, LOH of the same region was detected (11M between D3S1314 and D3S1311). In other cases, the pattern of LOH in the endometriosis was more discrete than in the carcinoma (18542, LOH of both D11S4046 and D11S4191 in the carcinoma, but only in D11S4046 in the endometriosis). L, loss of heterozygosity; H, heterozygous (retention of heterozygosity); N, noninformative; –, failed.

triosis and carcinoma, in all the common 22 LOH events combined is therefore very small ($p = 2.38 \times 10^{-7}$). In addition, this calculation has taken into account neither the probability of LOH occurring at the same locus in the endometriosis and cancer, nor that the regions of LOH in at least one case appeared to be the same (sample 11M between D3S1614 and D3S1311). The 2 cases where the endometriosis was located adjacent to the tumor (1 case of endometrioid (18542) and 1 case of clear cell ovarian cancer (11M)) showed the highest number of common LOH levels, but even case 914, where the endometriosis and tumor were in separate ovaries, showed 3 events in common (Table II). We conclude that endometriosis is likely to be a genetic precursor to EAOC in these 8 cases.

Discussion

In order to search for evidence of clonal progression, we analyzed 10 EAOC cases with coexisting endometriosis for common molecular genetic alterations. Using 82 microsatellite markers spanning the genome, we detected 63 LOH events in the carcinoma samples; twenty two of these were also detected in the corresponding endometriosis sample, involving the same allele in each case. Interestingly, no marker showed LOH in the endometriosis alone. Statistical analyses suggest that it is very unlikely that these common genetic alterations have occurred as independent events, and therefore we have provided evidence that endometriosis can be a clonal precursor to EAOCs.

Although endometriosis is a ''benign'' disease, it has many characteristics of neoplasia.[17] Endometriosis may show unrestrained growth and increased vascularization, and even features classically associated with malignancy, such as tissue invasion and metastasis. By detecting LOH in endometriosis lesions, we have also provided molecular evidence to suggest that endometriosis lesions can be true neoplasms, that is, they derive from a clonal proliferation of cells arising from genetic changes, which provide that clone with a replicative advantage.

There have only been a few LOH studies analyzing solitary endometriosis and EAOC.[23–29] For example, Jiang *et al*. (1998) ana-

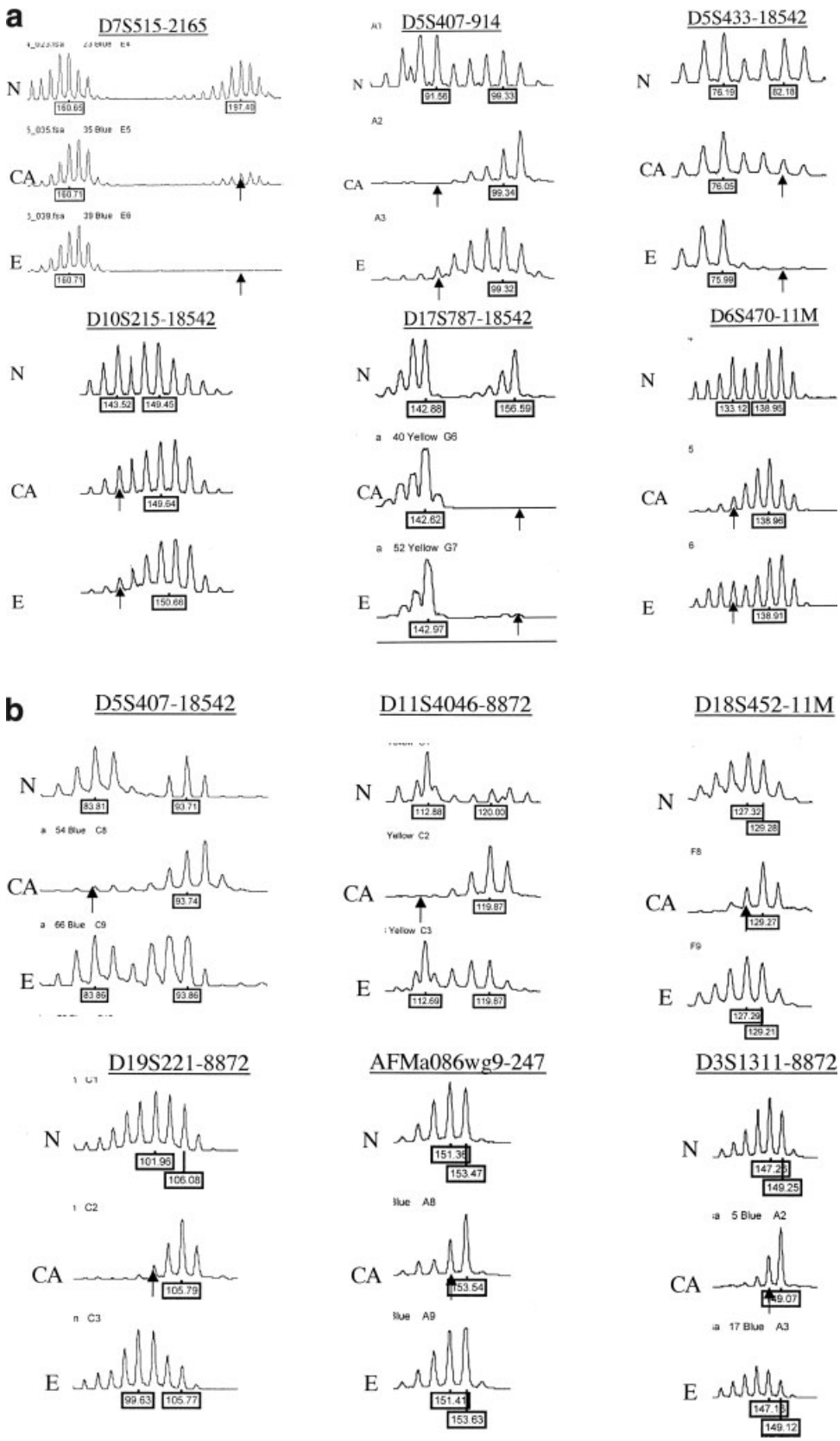

FIGURE 2 – (*a*) LOH at markers D7S515, D5S407, D5S433, D10S-215, D17S787 and D6S470 in both ovarian carcinoma and corresponding endometriosis from cases 2165, 914, 18542, 18542, 18542 and 11M respectively. (*b*) LOH at markers D5S407, D11S4046, D18S452, D19S221, AFMa086wg9 and D3S1311 in the carcinoma alone, with retention in the corresponding endometriosis, in cases 18542, 8872, 11M, 8872, 247 and 8872, respectively. Representative electropherograms from the analysis of normal ovarian stroma (N), carcinoma (CA) and coexisting endometriosis (E) are shown. The arrow indicates the 'lost' allele in each case. Sizes of the alleles, in base pairs, are indicated under the allele peak.

lyzed 14 cases of endometriosis synchronous with endometrioid ovarian cancer for LOH on 12 chromosome arms, using 25 markers.[25] In 9/11 cases where the carcinoma had either arisen within, or was adjacent to the endometriosis, LOH of the same allele was detected. However, none of the 3 cases where the endometriosis and carcinoma were from contralateral ovaries displayed

any common LOH, and in one of these cases LOH was detected in the endometriosis and not in the carcinoma. However, the majority of LOH studies have been limited by a number of factors, including (*i*) the small number of markers analyzed (*ii*) not using laser microdissection and (*iii*) nonquantitative LOH analysis from autoradiographs. We have improved upon these studies, reducing the potential for artifacts, by using fine scale microdissection and quantitative, fluorescent-based detection of LOH. The sample size in our study, and others, is small: Histopathologists diagnosing ovarian cancer often do not look for, or note, the presence of endometriosis, and therefore the samples are hard to obtain. However, by using a larger panel of markers, we were able to detect many more LOH events common to both the carcinoma and endometriosis, providing more convincing evidence of a common lineage and demonstrating that common allele loss is not a chromosome-specific event. In addition, to our knowledge, this study is the first to show evidence that endometriosis can be a clonal precursor to (*i*) clear cell, as well as endometrioid ovarian cancer and, (*ii*) carcinomas from contralateral ovaries, as well as cases where the endometriosis is adjacent to the carcinoma.

The frequency of malignant transformation in ovarian endometriosis is currently uncertain. Large pathology series have found ovarian cancer in 5–10% of endometriomas,[6,14] and malignant transformation of endometriosis has been estimated as 0.7–1.6% over an average of 8 years.[30,31] Other studies have found endometriosis in 21–54% of endometrioid and clear cell ovarian carcinomas,[5,11,13,32,33] but this is likely to be an underestimate, as the cancer can obliterate any endometriosis present. It may well be that the majority, if not all, of endometrioid and clear cell ovarian carcinomas originally derive from endometriosis,[2,25,34] although there is currently no evidence for this.

Some studies have suggested that EAOCs may be a separate entity from other ovarian carcinomas, where patients with EAOC are generally premenopausal, and have early stage disease and better survival.[8,35] These clinical observations could be explained by the fact that the carcinoma may be detected at an earlier stage because of endometriosis-associated symptoms, or that the endometriosis could be obliterated by the time the disease has progressed to the higher stages. In addition, high stage EAOCs have been documented. All our samples were low stage, and it would certainly be interesting to analyze high stage tumors coexisting with endometriosis to determine whether there were common molecular alterations. Since the two diseases share the same predisposing factors, and biological consequences of endometriosis, such as infertility, are associated with increased risks of ovarian cancer, we cannot conclude that endometriosis is a genetic precursor to EAOCs in all cases. Unfortunately, we do not have data as to whether our patients had a long history of infertility or not. Clearly, however, future studies to identify genes involved in EAOCs will have significance in the early development of at least a subset of endometrioid and clear cell ovarian cancers.

The ultimate aim of our studies is to identify genes that are involved in the development of endometriosis and its progression to malignancy. It has been shown that LOH is selected for, and therefore are detected at high frequencies, when it is involved in the inactivation of tumor suppressor genes. In our study, LOH was generally detected at low frequencies; however, D5S407 at 5q11.2, D6S470 at 6p24.3 and D13S265 at 13q31.2-q31.3 showed LOH in 2 endometriosis/tumor pairs and D17S787 at 17q22-23.2 showed LOH in 3 endometriosis/carcinoma samples, and therefore these loci may contain candidate tumor suppressor genes involved in the initiation of EAOCs. Our findings suggest that larger future studies using a similar strategy with a higher density of polymorphic markers will provide an opportunity to identify loci involved in clonal progression of endometriosis to cancer.

An increased understanding of the molecular events involved in the initiation and development of EAOC will provide a basis for developing novel forms of early diagnosis and therapy. Our findings have provided convincing evidence that endometriosis can be a precursor to EAOCs and further studies are needed to establish how molecular markers of progression can be used to identify women with endometriosis at highest risk of EAOC.

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