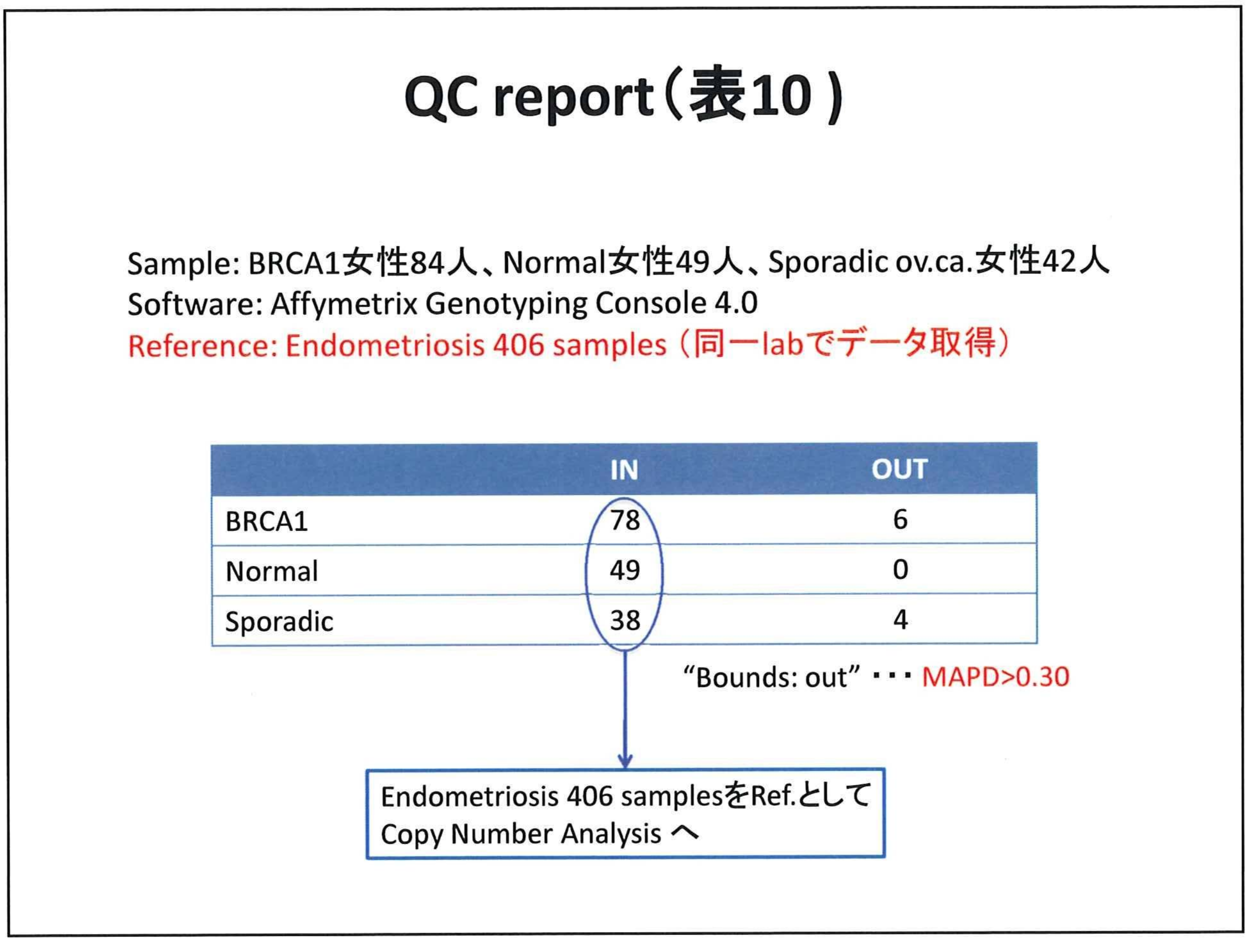


QC report（表10)

Sample: BRCA1女性84人、Normal女性49人、Sporadic ov.ca.女性42人
Software: Affymetrix Genotyping Console 4.0
Reference: Endometriosis 406 samples（同一labでデータ取得）

	IN	OUT
BRCA1	78	6
Normal	49	0
Sporadic	38	4

"Bounds: out" ･･･ MAPD>0.30

Endometriosis 406 samplesをRef.として
Copy Number Analysis へ

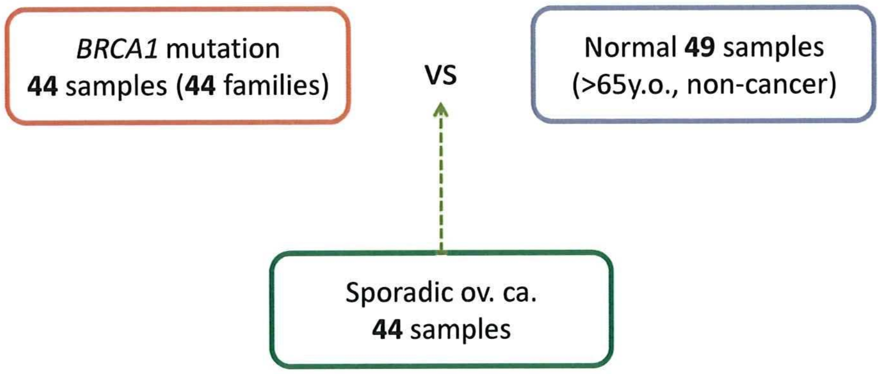
（表11）BRCA1変異陽性症例に特異的なコピー数変化の同定

1．Software: Partek Genomic Suite v6.4

2．Reference (Baseline): HapMap JPT female 21 samples

3．アルゴリズム: Hidden Markov Model
(genomic marker >3、Copy Number Variation > 1kb Nature Rev Genet 2006)

【1家系に対し1サンプル】

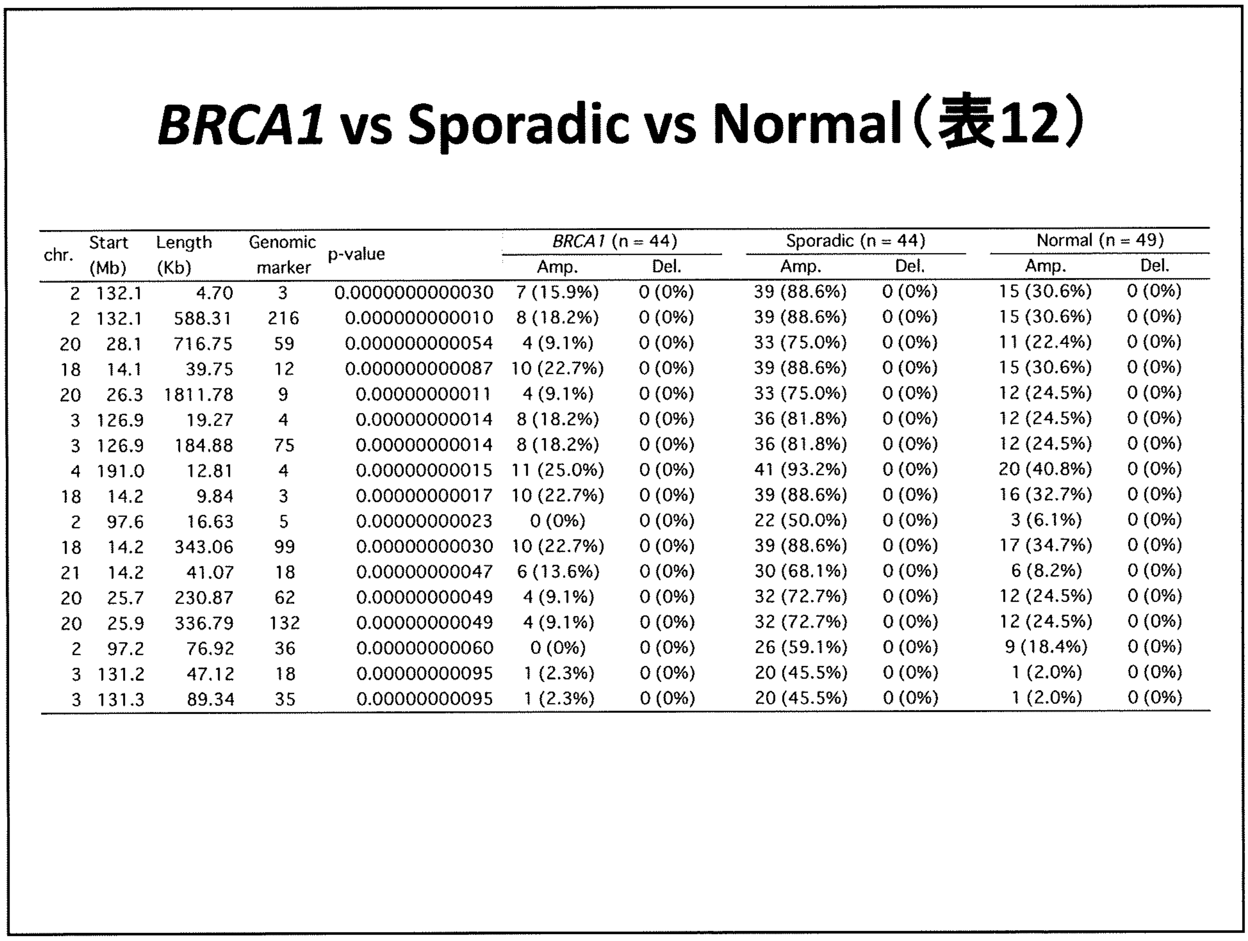


BRCA1 mutation
44 samples (**44** families)

VS

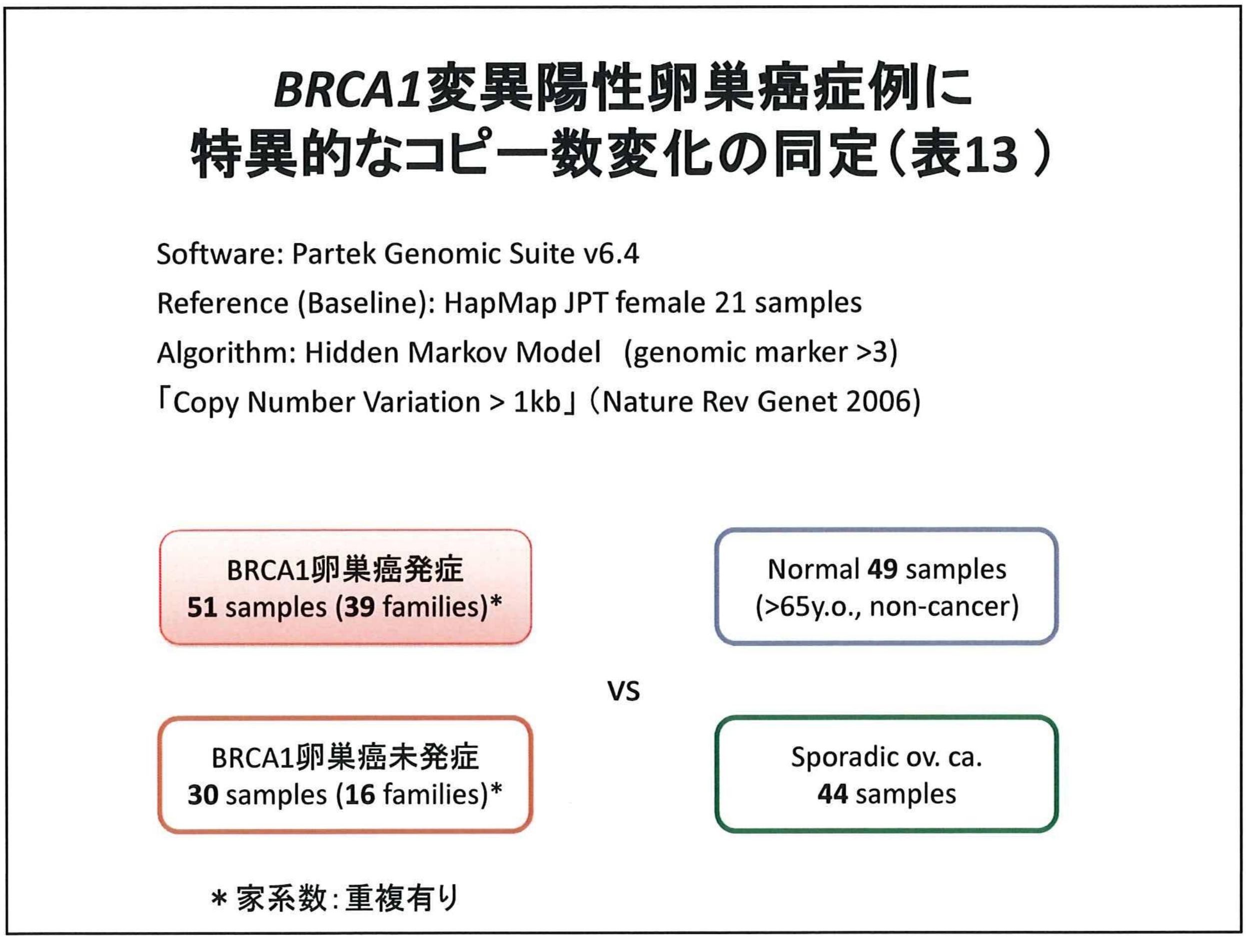
Normal **49** samples
(>65y.o., non-cancer)

Sporadic ov. ca.
44 samples



BRCA1 vs Sporadic vs Normal（表12）

chr.	Start (Mb)	Length (Kb)	Genomic marker	p-value	*BRCA1* (n = 44)		Sporadic (n = 44)		Normal (n = 49)	
					Amp.	Del.	Amp.	Del.	Amp.	Del.
2	132.1	4.70	3	0.0000000000030	7 (15.9%)	0 (0%)	39 (88.6%)	0 (0%)	15 (30.6%)	0 (0%)
2	132.1	588.31	216	0.000000000010	8 (18.2%)	0 (0%)	39 (88.6%)	0 (0%)	15 (30.6%)	0 (0%)
20	28.1	716.75	59	0.000000000054	4 (9.1%)	0 (0%)	33 (75.0%)	0 (0%)	11 (22.4%)	0 (0%)
18	14.1	39.75	12	0.000000000087	10 (22.7%)	0 (0%)	39 (88.6%)	0 (0%)	15 (30.6%)	0 (0%)
20	26.3	1811.78	9	0.00000000011	4 (9.1%)	0 (0%)	33 (75.0%)	0 (0%)	12 (24.5%)	0 (0%)
3	126.9	19.27	4	0.00000000014	8 (18.2%)	0 (0%)	36 (81.8%)	0 (0%)	12 (24.5%)	0 (0%)
3	126.9	184.88	75	0.00000000014	8 (18.2%)	0 (0%)	36 (81.8%)	0 (0%)	12 (24.5%)	0 (0%)
4	191.0	12.81	4	0.00000000015	11 (25.0%)	0 (0%)	41 (93.2%)	0 (0%)	20 (40.8%)	0 (0%)
18	14.2	9.84	3	0.00000000017	10 (22.7%)	0 (0%)	39 (88.6%)	0 (0%)	16 (32.7%)	0 (0%)
2	97.6	16.63	5	0.00000000023	0 (0%)	0 (0%)	22 (50.0%)	0 (0%)	3 (6.1%)	0 (0%)
18	14.2	343.06	99	0.00000000030	10 (22.7%)	0 (0%)	39 (88.6%)	0 (0%)	17 (34.7%)	0 (0%)
21	14.2	41.07	18	0.00000000047	6 (13.6%)	0 (0%)	30 (68.1%)	0 (0%)	6 (8.2%)	0 (0%)
20	25.7	230.87	62	0.00000000049	4 (9.1%)	0 (0%)	32 (72.7%)	0 (0%)	12 (24.5%)	0 (0%)
20	25.9	336.79	132	0.00000000049	4 (9.1%)	0 (0%)	32 (72.7%)	0 (0%)	12 (24.5%)	0 (0%)
2	97.2	76.92	36	0.00000000060	0 (0%)	0 (0%)	26 (59.1%)	0 (0%)	9 (18.4%)	0 (0%)
3	131.2	47.12	18	0.00000000095	1 (2.3%)	0 (0%)	20 (45.5%)	0 (0%)	1 (2.0%)	0 (0%)
3	131.3	89.34	35	0.00000000095	1 (2.3%)	0 (0%)	20 (45.5%)	0 (0%)	1 (2.0%)	0 (0%)



*BRCA1*変異陽性卵巣癌症例に特異的なコピー数変化の同定（表13 ）

Software: Partek Genomic Suite v6.4

Reference (Baseline): HapMap JPT female 21 samples

Algorithm: Hidden Markov Model (genomic marker >3)

「Copy Number Variation > 1kb」（Nature Rev Genet 2006)

BRCA1卵巣癌発症
51 samples (39 families)*

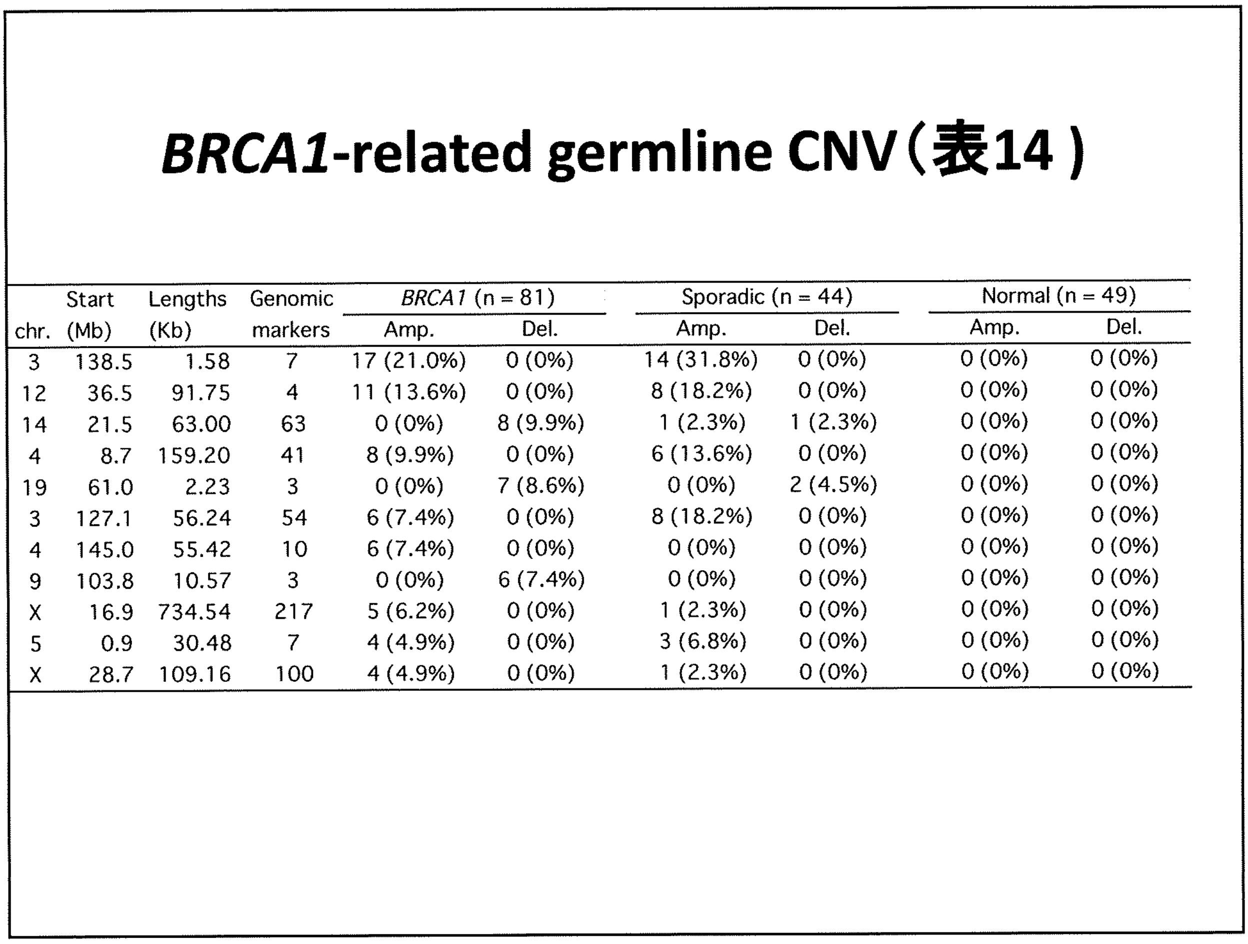
BRCA1卵巣癌未発症
30 samples (**16** families)*

VS

Normal **49** samples
(>65y.o., non-cancer)

Sporadic ov. ca.
44 samples

＊家系数：重複有り

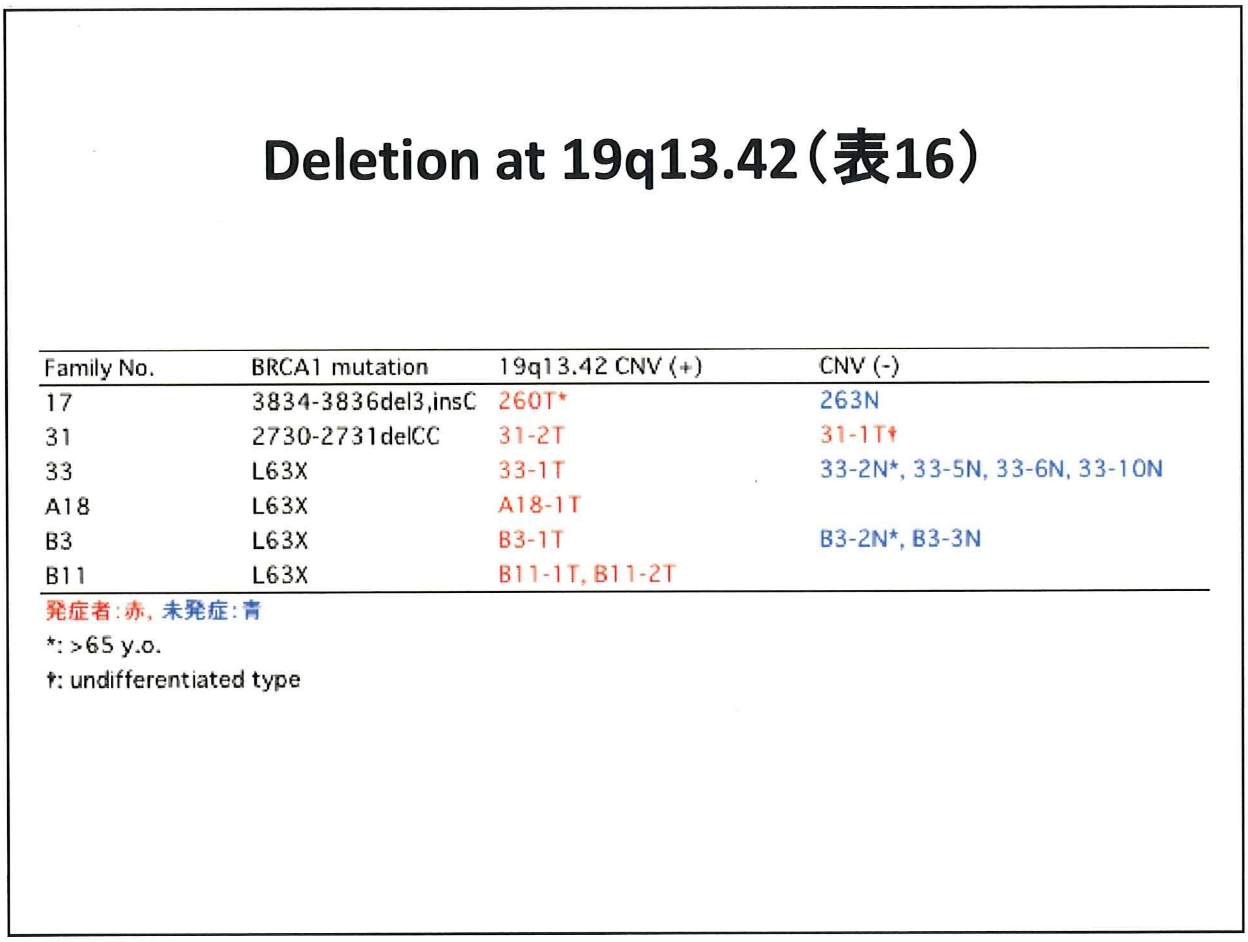


BRCA1-related germline CNV（表14）

	Start	Lengths	Genomic	*BRCA1* (n = 81)		Sporadic (n = 44)		Normal (n = 49)	
chr.	(Mb)	(Kb)	markers	Amp.	Del.	Amp.	Del.	Amp.	Del.
3	138.5	1.58	7	17 (21.0%)	0 (0%)	14 (31.8%)	0 (0%)	0 (0%)	0 (0%)
12	36.5	91.75	4	11 (13.6%)	0 (0%)	8 (18.2%)	0 (0%)	0 (0%)	0 (0%)
14	21.5	63.00	63	0 (0%)	8 (9.9%)	1 (2.3%)	1 (2.3%)	0 (0%)	0 (0%)
4	8.7	159.20	41	8 (9.9%)	0 (0%)	6 (13.6%)	0 (0%)	0 (0%)	0 (0%)
19	61.0	2.23	3	0 (0%)	7 (8.6%)	0 (0%)	2 (4.5%)	0 (0%)	0 (0%)
3	127.1	56.24	54	6 (7.4%)	0 (0%)	8 (18.2%)	0 (0%)	0 (0%)	0 (0%)
4	145.0	55.42	10	6 (7.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
9	103.8	10.57	3	0 (0%)	6 (7.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
X	16.9	734.54	217	5 (6.2%)	0 (0%)	1 (2.3%)	0 (0%)	0 (0%)	0 (0%)
5	0.9	30.48	7	4 (4.9%)	0 (0%)	3 (6.8%)	0 (0%)	0 (0%)	0 (0%)
X	28.7	109.16	100	4 (4.9%)	0 (0%)	1 (2.3%)	0 (0%)	0 (0%)	0 (0%)

BRCA1 ovarian cancer-related CNV（表15)

chr.	Start (Mb)	Lengths (Kb)	Genomic markers	*BRCA1* affected unchanged	*BRCA1* affected Amp.	*BRCA1* affected Deletion	*BRCA1* unaffected changed	Normal changed	Sporadic changed
3	138.5	1.58	7	38	13	0	4	0	14
19	61.0	2.23	3	44	0	7	0	0	2
12	36.5	91.75	4	46	5	0	6	0	8
3	127.1	2.50	4	46	5	0	2	0	8
4	145.0	55.42	10	46	5	0	1	0	0
9	103.8	10.57	3	47	0	4	2	0	0
3	127.1	56.24	54	47	4	0	2	0	7
5	0.9	30.48	7	47	4	0	0	0	3
14	21.5	63.00	63	47	0	4	4	0	1/1
4	8.7	159.20	41	47	4	0	4	0	6
X	16.9	734.54	217	47	4	0	1	0	1
X	28.7	109.16	100	47	4	0	0	0	1



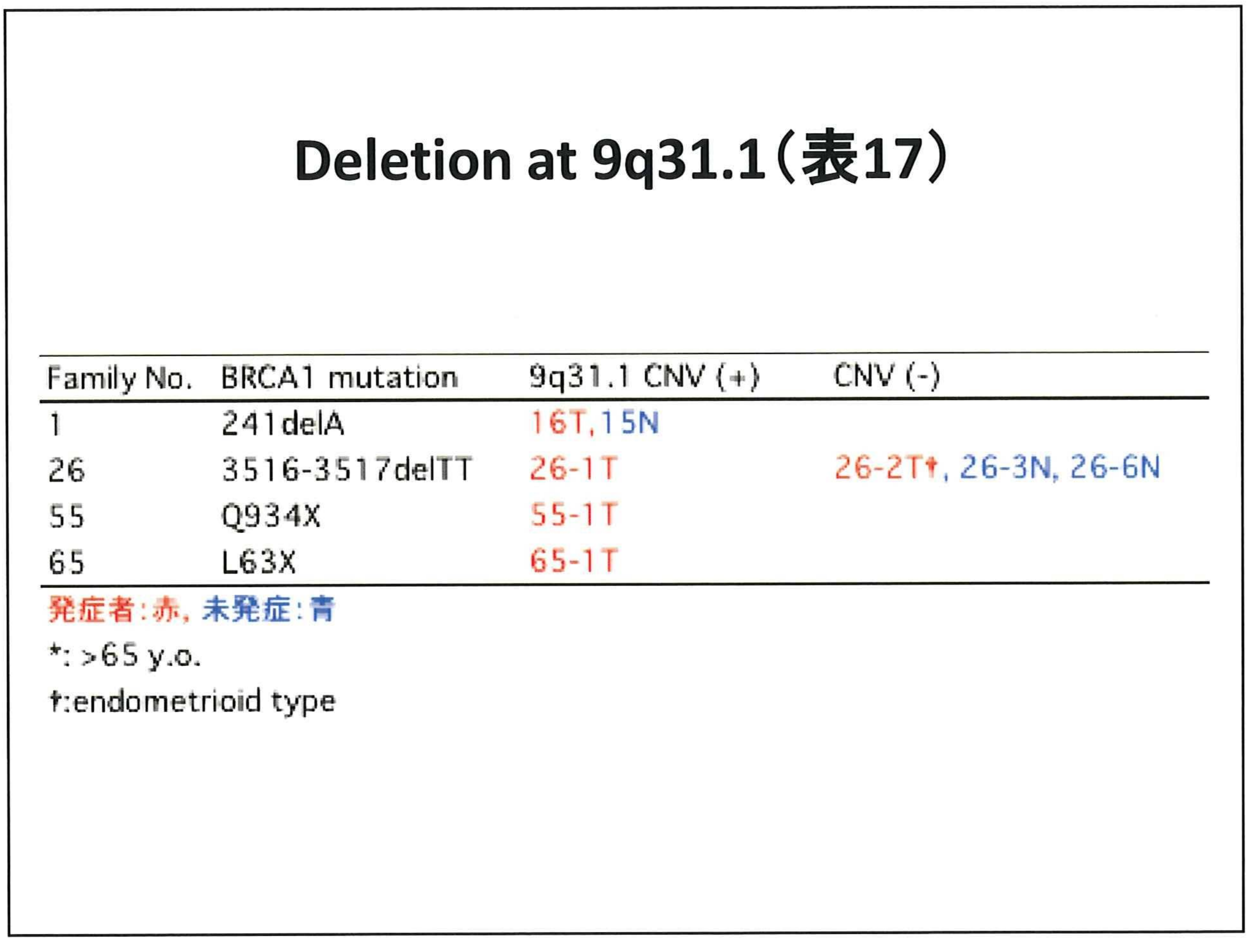
Deletion at 19q13.42（表16）

Family No.	BRCA1 mutation	19q13.42 CNV (+)	CNV (-)
17	3834-3836del3,insC	260T*	263N
31	2730-2731delCC	31-2T	31-1T†
33	L63X	33-1T	33-2N*, 33-5N, 33-6N, 33-10N
A18	L63X	A18-1T	
B3	L63X	B3-1T	B3-2N*, B3-3N
B11	L63X	B11-1T, B11-2T	

発症者：赤，未発症：青

*: >65 y.o.

†: undifferentiated type



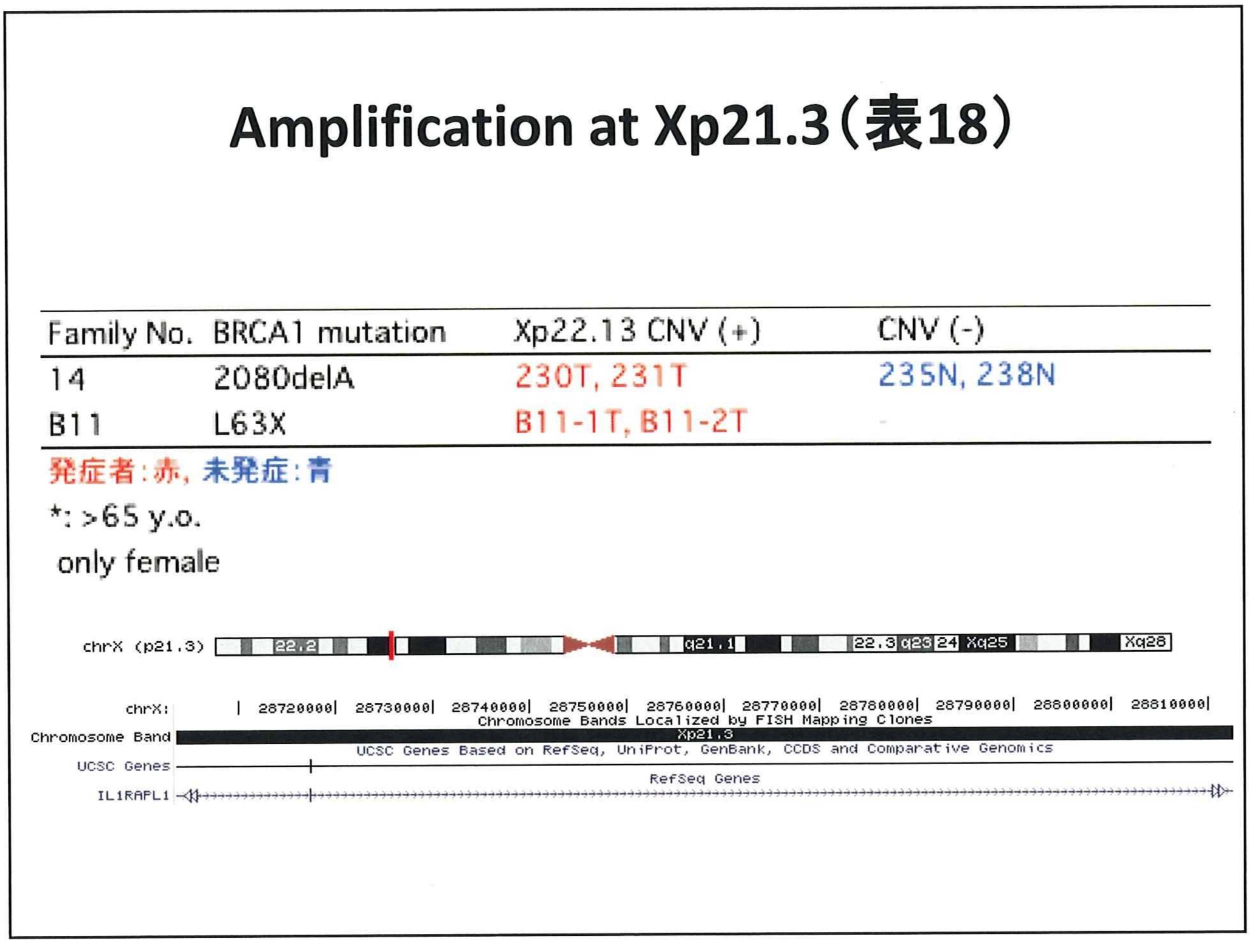
Deletion at 9q31.1（表17）

Family No.	BRCA1 mutation	9q31.1 CNV (+)	CNV (-)
1	241delA	16T, 15N	
26	3516-3517delTT	26-1T	26-2T†, 26-3N, 26-6N
55	Q934X	55-1T	
65	L63X	65-1T	

発症者:赤, 未発症:青

*: >65 y.o.

†:endometrioid type



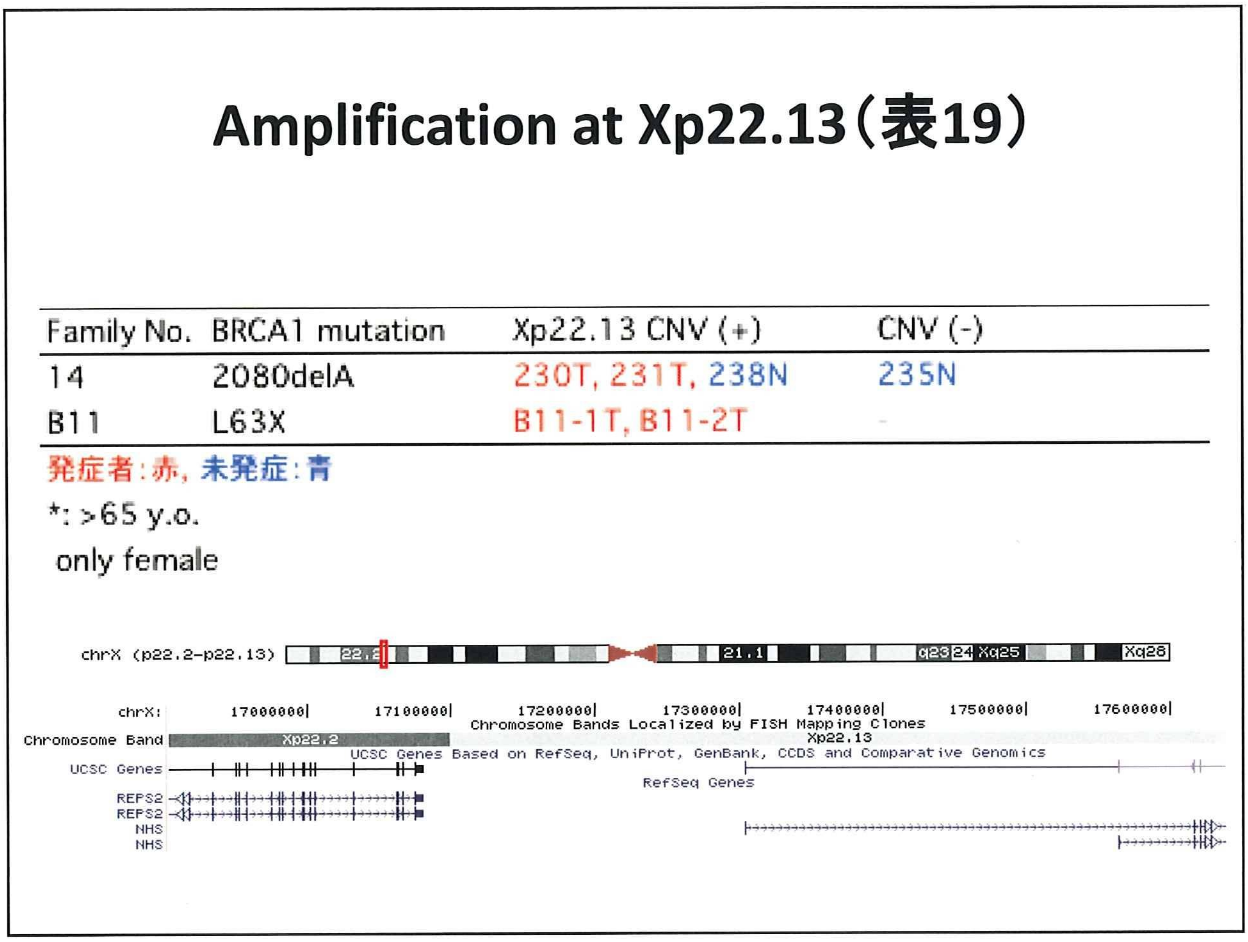
Amplification at Xp21.3（表18）

Family No.	BRCA1 mutation	Xp22.13 CNV (+)	CNV (-)
14	2080delA	230T, 231T	235N, 238N
B11	L63X	B11-1T, B11-2T	-

発症者:赤, 未発症:青

*: >65 y.o.

only female



Amplification at Xp22.13（表19）

Family No.	BRCA1 mutation	Xp22.13 CNV (+)	CNV (-)
14	2080delA	230T, 231T, 238N	235N
B11	L63X	B11-1T, B11-2T	-

発症者:赤, 未発症:青

*: >65 y.o.

only female

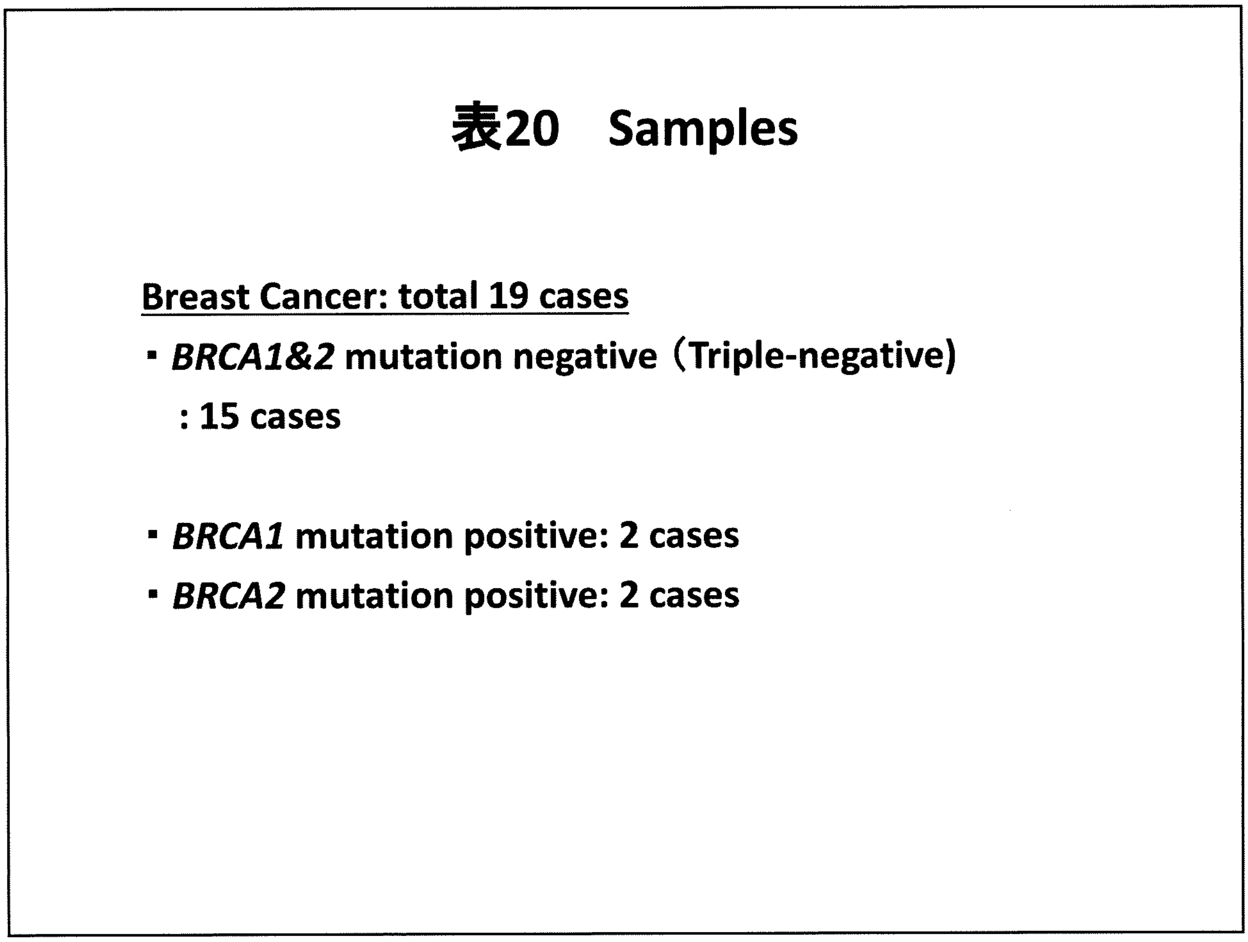


表20　Samples

<u>Breast Cancer: total 19 cases</u>

- ***BRCA1&2*** **mutation negative（Triple-negative)**
  : 15 cases

- ***BRCA1*** **mutation positive: 2 cases**
- ***BRCA2*** **mutation positive: 2 cases**

表21　Frequently observed copy number gains and losses(Breast Cancer)

Chromosome	Freq (%)			Observed region	
	Overall	Tripple-Negative	*BRCA*1/2 mutaion	Size (Mb)	Average CN
Gain					
1q	**42.1**	**40.0**	**50.0**	**2.82**	**2.9**
8q	36.8	33.3	50.0	0.88	3.4
17q	36.8	26.7	48.0	0.11	2.9

Copy number alterations with >40% frequency and >0.1Mb size

表22

Ovarian cancer-specific copy number alterations

Materials: T/N-Paired samples (n = 43)
  Sporadic ovarian cancer: 33 samples
  Familial ovarian cancer: 10 samples
Method: Affymetrix Genome-Wide Human SNP Array 5.0

Ovarian cancer-specific gene expression profiles

Samples: Serous ovarian cancer (n = 34)
  Sporadic ovarian cancer: 31 samples
  Familial ovarian cancer: 3 samples
Method: Agilent Human 1A v2 oligonucleotide microarray

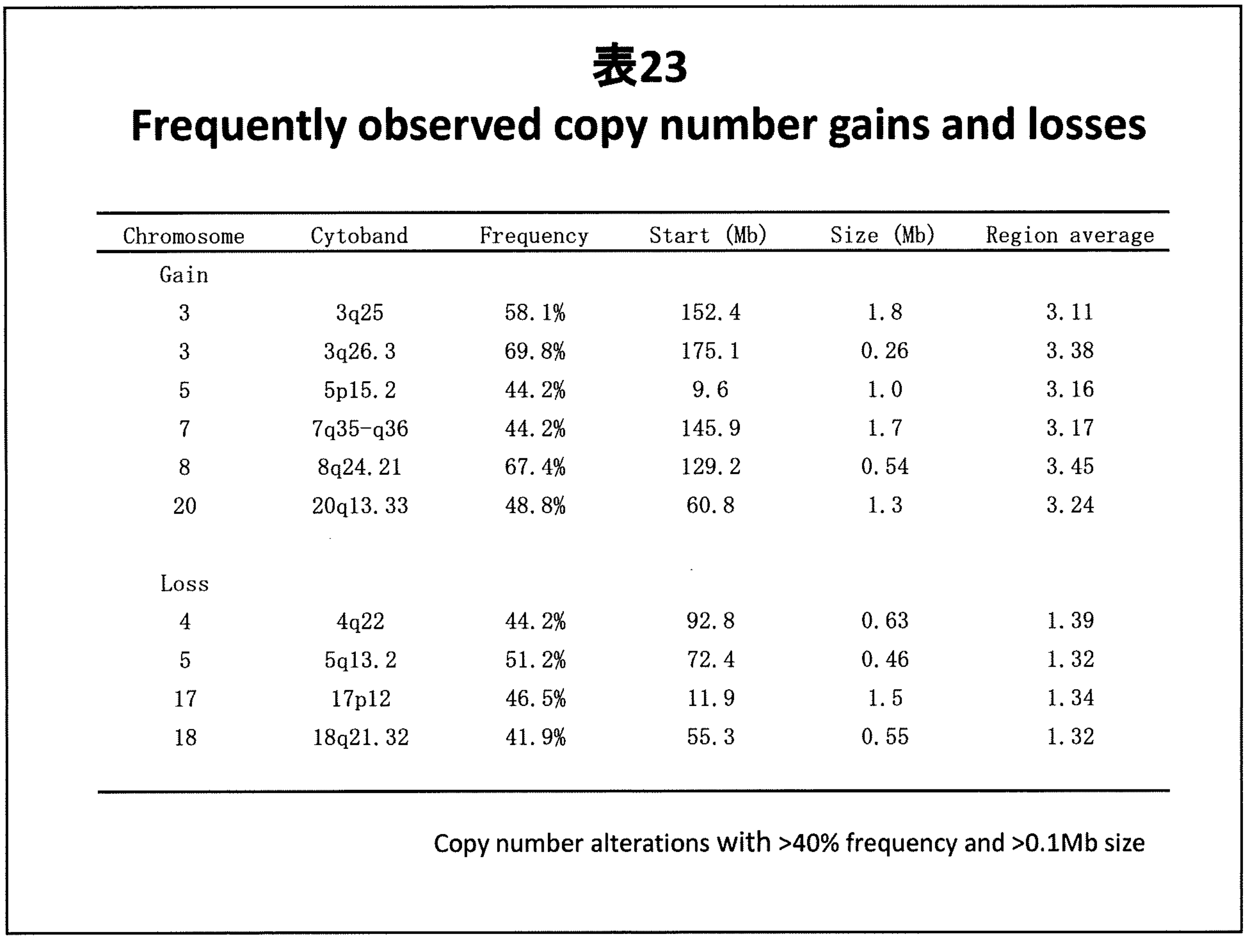


表23
Frequently observed copy number gains and losses

Chromosome	Cytoband	Frequency	Start (Mb)	Size (Mb)	Region average
Gain					
3	3q25	58.1%	152.4	1.8	3.11
3	3q26.3	69.8%	175.1	0.26	3.38
5	5p15.2	44.2%	9.6	1.0	3.16
7	7q35-q36	44.2%	145.9	1.7	3.17
8	8q24.21	67.4%	129.2	0.54	3.45
20	20q13.33	48.8%	60.8	1.3	3.24
Loss					
4	4q22	44.2%	92.8	0.63	1.39
5	5q13.2	51.2%	72.4	0.46	1.32
17	17p12	46.5%	11.9	1.5	1.34
18	18q21.32	41.9%	55.3	0.55	1.32

Copy number alterations with >40% frequency and >0.1Mb size

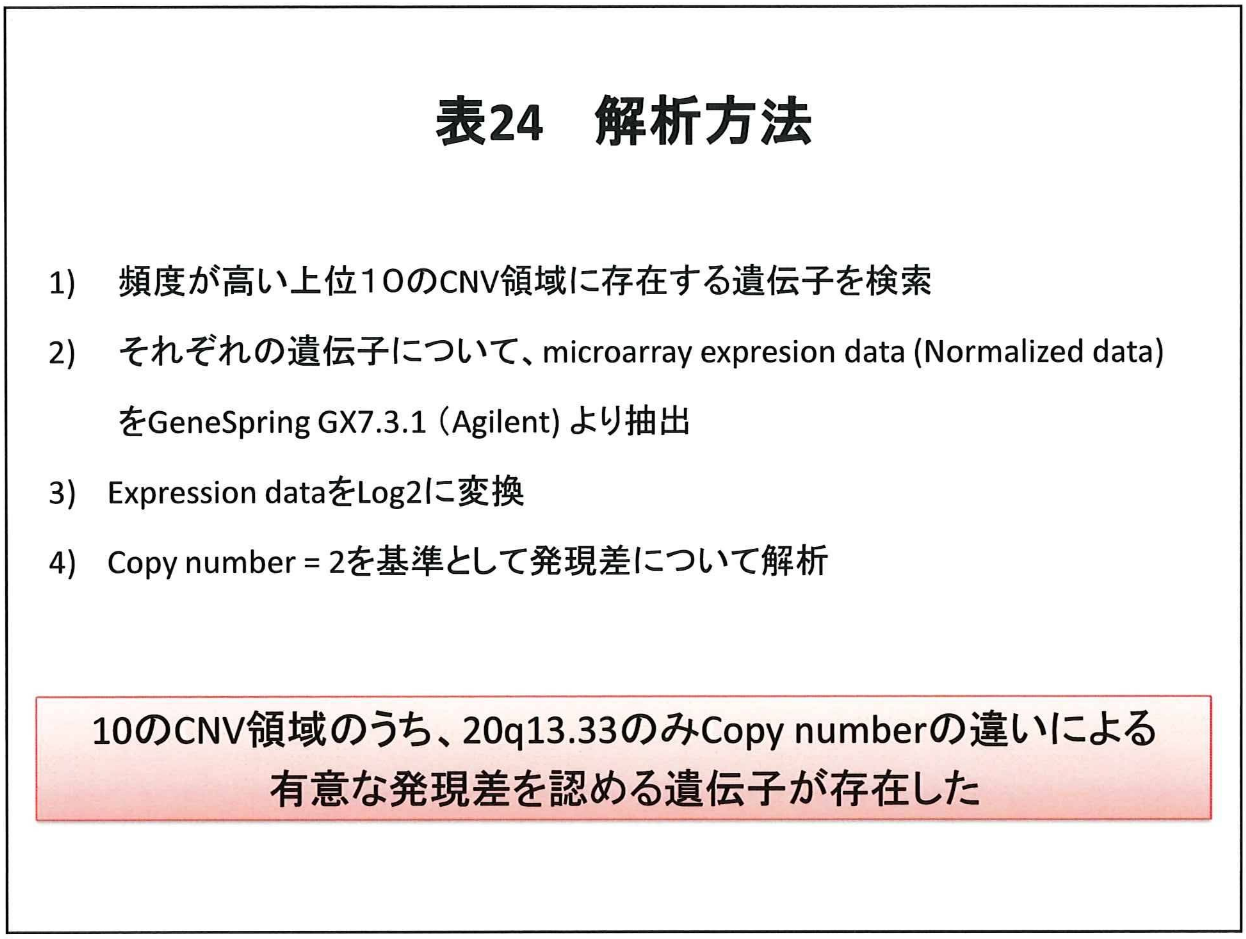


表24　解析方法

1) 頻度が高い上位10のCNV領域に存在する遺伝子を検索
2) それぞれの遺伝子について、microarray expresion data (Normalized data) をGeneSpring GX7.3.1 (Agilent) より抽出
3) Expression dataをLog2に変換
4) Copy number = 2を基準として発現差について解析

10のCNV領域のうち、20q13.33のみCopy numberの違いによる有意な発現差を認める遺伝子が存在した

表25　Gene Expression (Copy number 2 vs >3)

Gene Symbol	location	CN=2 (n=17) Expression (mean)	CN≧3(n=17) Expression (mean)	p-value*
C20orf11	20q13.33	3.970	4.816	0.0007
C20orf149	20q13.33	6.795	7.556	0.0080
C20orf20	20q13.33	2.520	2.943	0.0098
COL9A3	20q13.33	1.189	3.402	0.0145
DIDO1	20q13.33	0.359	1.480	<0.0001
ZNF512B	20q13.33	3.282	4.132	0.0003
LIME1	20q13.33	0.230	0.628	0.0357
PRPF6	20q13.33	5.025	5.689	0.0002
SLC2A4RG	20q13.33	6.016	6.746	0.0022
STMN3	20q13.33	4.402	5.571	0.0458
TCFL5	20q13.33	1.132	1.811	0.0007
UCKL1	20q13.33	3.811	4.645	0.0001
YTHDF1	20q13.33	5.151	6.015	0.0002
ARFGAP1	20q13.33	4.485	5.180	0.0011

*Mann-Whitney test

表26
Gene Expression (Copy number 2 vs >3)

	Expression mean data (Log2)				Dunn’s Multiple Comparison Test		
Gene Symbol	CN=2 (n=17)	CN=3 (n=14)	CN=4 (n=3)	p-value**	2 vs 3	2 vs 4	3 vs 4
C20orf11	3.970	4.741	4.939	0.0016	$P < 0.05$	$P < 0.05$	$P > 0.05$
C20orf149	6.795	7.390	8.332	0.0081	$P < 0.05$	$P < 0.05$	$P > 0.05$
C20orf20	2.520	2.903	3.131	0.0264	$P > 0.05$	$P > 0.05$	$P > 0.05$
COL9A3	1.189	3.067	4.961	0.0325	$P > 0.05$	$P > 0.05$	$P > 0.05$
DIDO1	0.359	1.413	1.794	<0.0001	$P < 0.001$	$P < 0.01$	$P > 0.05$
ZNF512B	3.282	4.031	4.603	0.0009	$P < 0.01$	$P > 0.05$	$P > 0.05$
LIME1	0.637	0.960	1.624	0.0483	$P > 0.05$	$P > 0.05$	$P > 0.05$
PRPF6	5.025	5.605	6.083	0.0005	$P < 0.01$	$P < 0.01$	$P > 0.05$
SLC2A4RG	6.016	6.616	7.354	0.0030	$P < 0.05$	$P < 0.05$	$P > 0.05$
STMN3	4.402	5.350	6.600	0.0719	–	–	–
TCFL5	1.132	1.645	2.584	0.0021	$P < 0.01$	$P < 0.05$	$P > 0.05$
UCKL1	3.811	4.524	5.209	0.0004	$P < 0.01$	$P < 0.01$	$P > 0.05$
YTHDF1	5.151	5.903	6.539	0.0004	$P < 0.01$	$P < 0.01$	$P > 0.05$
ARFGAP1	4.485	5.002	6.015	0.0012	$P < 0.05$	$P < 0.01$	$P > 0.05$

**Kruskal-Wallis test

表27 Clinical Characteristics (Copy number 2 vs >3)

	Copy Numver 2(n=17)	Copy Number>3(n=17)	p-value
BRCA1 status			1.0000**
mutated	2	1	
wild type	15	16	
Age (mean±SD)	55.24±2.943	61.71±2.318	0.0937*
Stage			
stage I/II	5	5	1.0000**
stage III/IV	12	12	
Surgery			
optimal (<1cm)	12	13	1.0000**
not optimal	5	4	
LN metastasis			
positive	4	2	0.6539**
negative	12	14	
Ascitic fluid cytology			
positive	12	13	1.0000**
negative	5	4	
Grade			
low (Grade1)	7	6	1.0000**
high (Grade2/3)	10	11	

* unpaired t-test, **Fisher's exact test, ***Chi-square test

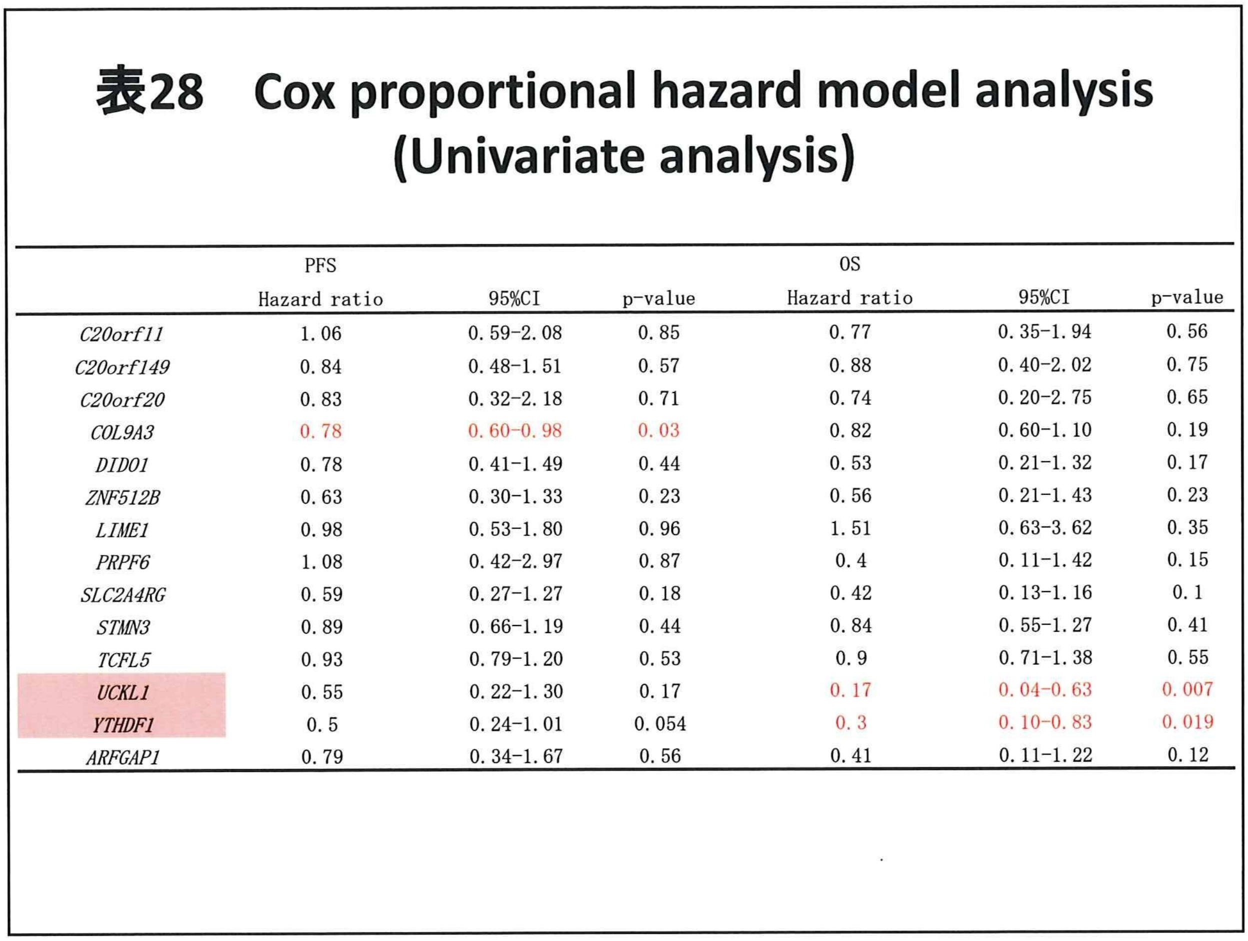


表28　Cox proportional hazard model analysis (Univariate analysis)

	PFS Hazard ratio	95%CI	p-value	OS Hazard ratio	95%CI	p-value
C20orf11	1. 06	0. 59-2. 08	0. 85	0. 77	0. 35-1. 94	0. 56
C20orf149	0. 84	0. 48-1. 51	0. 57	0. 88	0. 40-2. 02	0. 75
C20orf20	0. 83	0. 32-2. 18	0. 71	0. 74	0. 20-2. 75	0. 65
COL9A3	0. 78	0. 60-0. 98	0. 03	0. 82	0. 60-1. 10	0. 19
DIDO1	0. 78	0. 41-1. 49	0. 44	0. 53	0. 21-1. 32	0. 17
ZNF512B	0. 63	0. 30-1. 33	0. 23	0. 56	0. 21-1. 43	0. 23
LIME1	0. 98	0. 53-1. 80	0. 96	1. 51	0. 63-3. 62	0. 35
PRPF6	1. 08	0. 42-2. 97	0. 87	0. 4	0. 11-1. 42	0. 15
SLC2A4RG	0. 59	0. 27-1. 27	0. 18	0. 42	0. 13-1. 16	0. 1
STMN3	0. 89	0. 66-1. 19	0. 44	0. 84	0. 55-1. 27	0. 41
TCFL5	0. 93	0. 79-1. 20	0. 53	0. 9	0. 71-1. 38	0. 55
UCKL1	0. 55	0. 22-1. 30	0. 17	0. 17	0. 04-0. 63	0. 007
YTHDF1	0. 5	0. 24-1. 01	0. 054	0. 3	0. 10-0. 83	0. 019
ARFGAP1	0. 79	0. 34-1. 67	0. 56	0. 41	0. 11-1. 22	0. 12

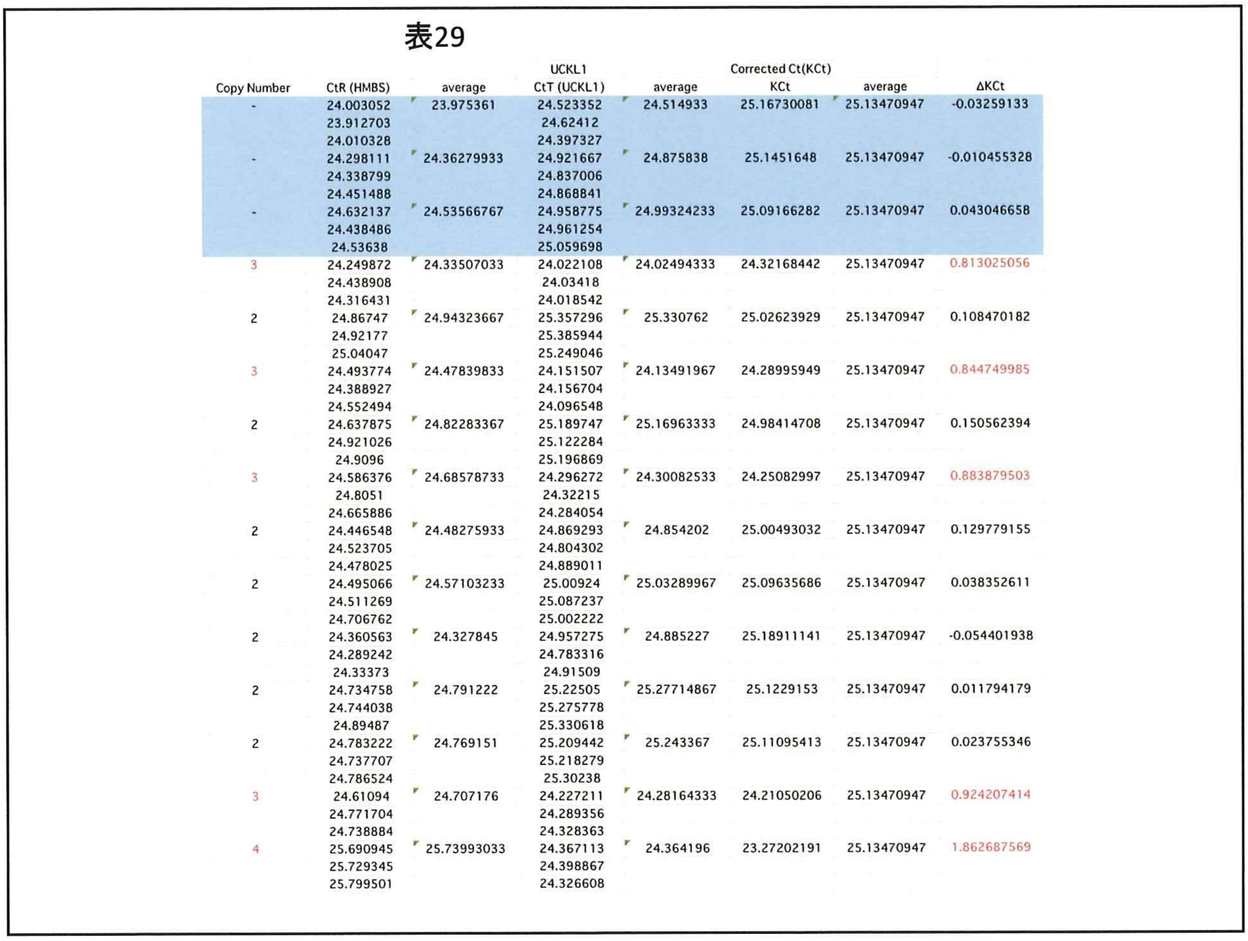


表29

			UCKL1		Corrected Ct(KCt)		
Copy Number	CtR (HMBS)	average	CtT (UCKL1)	average	KCt	average	ΔKCt
-	24.003052	23.975361	24.523352	24.514933	25.16730081	25.13470947	-0.03259133
	23.912703		24.62412				
	24.010328		24.397327				
-	24.298111	24.36279933	24.921667	24.875838	25.1451648	25.13470947	-0.010455328
	24.338799		24.837006				
	24.451488		24.868841				
-	24.632137	24.53566767	24.958775	24.99324233	25.09166282	25.13470947	0.043046658
	24.438486		24.961254				
	24.53638		25.059698				
3	24.249872	24.33507033	24.022108	24.02494333	24.32168442	25.13470947	0.813025056
	24.438908		24.03418				
	24.316431		24.018542				
2	24.86747	24.94323667	25.357296	25.330762	25.02623929	25.13470947	0.108470182
	24.92177		25.385944				
	25.04047		25.249046				
3	24.493774	24.47839833	24.151507	24.13491967	24.28995949	25.13470947	0.844749985
	24.388927		24.156704				
	24.552494		24.096548				
2	24.637875	24.82283367	25.189747	25.16963333	24.98414708	25.13470947	0.150562394
	24.921026		25.122284				
	24.9096		25.196869				
3	24.586376	24.68578733	24.296272	24.30082533	24.25082997	25.13470947	0.883879503
	24.8051		24.32215				
	24.665886		24.284054				
2	24.446548	24.48275933	24.869293	24.854202	25.00493032	25.13470947	0.129779155
	24.523705		24.804302				
	24.478025		24.889011				
2	24.495066	24.57103233	25.00924	25.03289967	25.09635686	25.13470947	0.038352611
	24.511269		25.087237				
	24.706762		25.002222				
2	24.360563	24.327845	24.957275	24.885227	25.18911141	25.13470947	-0.054401938
	24.289242		24.783316				
	24.33373		24.91509				
2	24.734758	24.791222	25.22505	25.27714867	25.1229153	25.13470947	0.011794179
	24.744038		25.275778				
	24.89487		25.330618				
2	24.783222	24.769151	25.209442	25.243367	25.11095413	25.13470947	0.023755346
	24.737707		25.218279				
	24.786524		25.30238				
3	24.61094	24.707176	24.227211	24.28164333	24.21050206	25.13470947	0.924207414
	24.771704		24.289356				
	24.738884		24.328363				
4	25.690945	25.73993033	24.367113	24.364196	23.27202191	25.13470947	1.862687569
	25.729345		24.398867				
	25.799501		24.326608				