学位論文

Integrated Analysis of DNA Methylation and Mutations in Esophageal Squamous Cell Carcinoma.

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Integrated Analysis of DNA Methylation and Mutations in Esophageal Squamous Cell Carcinoma

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The recent development of next-generation sequencing technology for extensive mutation analysis, and beadarray technology for genome-wide DNA methylation analysis has made it possible to obtain integrated pictures of genetic and epigenetic alterations, using the same cancer samples. In this study, we aimed to characterize such a picture in esophageal squamous cell carcinomas (ESCCs). Base substitutions of 55 cancer-related genes and copy number alterations (CNAs) of 28 cancer-related genes were analyzed by targeted sequencing. Forty-four of 57 ESCCs (77%) had 64 non-synonymous somatic mutations, and 24 ESCCs (42%) had 35 CNAs. A genome-wide DNA methylation analysis using an Infinium HumanMethylation450 BeadChip array showed that the CpG island methylator phenotype was unlikely to be present in ESCCs, a different situation from gastric and colon cancers. Regarding individual pathways affected in ESCCs, the WNT pathway was activated potentially by aberrant methylation of its negative regulators, such as *SFRP1*, *SFRP2*, *SFRP4*, *SFRP5*, *SOX17*, and *WIF1* (33%). The p53 pathway was inactivated by *TP53* mutations (70%), and potentially by aberrant methylation of its downstream genes. The cell cycle was deregulated by mutations of *CDKN2A* (9%), deletions of *CDKN2A* and *RB1* (32%), and by aberrant methylation of *CDKN2A* and *CHFR* (9%). In conclusion, ESCCs had unique methylation profiles different from gastric and colon cancers. The genes involved in the WNT pathway were affected mainly by epigenetic alterations, and those involved in the p53 pathway and cell cycle regulation were affected mainly by genetic alterations.

Key words: epigenetics; DNA methylation; genetic alterations; esophageal squamous cell carcinoma (ESCC); cancer-related pathway

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is one of the most malignant cancers worldwide, and a predominant histological type of esophageal cancer [1,2]. To understand ESCC development, many studies have been conducted showing that *TP53* is most frequently mutated (35–84%), and that other genes, such as *CDKN2A*, *CDKN2B*, *EGFR*, *NFE2L2*, *PIK3CA*, *MLH1*, and *BRAF*, are mutated with relatively low frequencies [3–7]. Recently, four comprehensive mutation analyses by whole-genome and whole-exome sequencing have been reported in ESCCs, and *ADAM29*, *AJUBA*, *ARID1A*, *FAM135B*, *FAT1*, *FAT2*, *FAT3*, *FAT4*, *KEAP1*, *KMT2D*, *NOTCH1*, *NOTCH2*, *NOTCH3*, and *ZNF750* were newly identified as mutated genes [8–11].

Epigenetic alterations, such as aberrant DNA methylation, are deeply involved in human cancer development [12–15]. Especially, aberrant DNA methylation of a CpG island (CGI) in a promoter region causes silencing of its downstream gene and is known as a major mechanism for inactivation of tumor-suppressor genes [16]. In ESCCs, *CDKN2A* has been reported to be methylated in 19–88% of cases [17–19], and its methylation is associated with metastatic and invasive phenotypes of ESCCs [20]. Additional genes, such as *APC*, *CDH1*, *CDKN2B*, and *RASSF1*, have also been reported to be methylated in ESCCs [21–26].

In this study, we aimed to establish an integrated picture of genetic and epigenetic alterations in ESCCs taking advantage of two novel technologies. Next-generation sequencing (NGS) technology has enabled

Abbreviations: NGS, next-generation sequencing; ESCC, esophageal squamous cell carcinoma; CGI, CpG island; CNV, copy number variation; RRDR, relative reading depth to the reference; array-CGH, microarray-based comparative genomic hybridization; TSS, transcription start site; TSS200, 200-bp upstream region from a transcription start site; OS, overall survival; dCRT, definitive chemoradiotherapy; CIMP, CpG island methylator phenotype.

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CGH + SNP 4 × 180 K microarray (Agilent Technologies). The microarray was scanned with an Agilent G2565BA microarray scanner (Agilent Technologies), and the scanned data were processed using the Agilent CytoGenomics Software (Agilent Technologies). A relative copy number of a gene was obtained as two to the power of the mean log2 ratio of all probes within the gene.

Analysis of Gene Expression by Oligonucleotide Microarray

Gene expression levels in normal esophageal mucosae and ESCC cell lines (KYSE30, KYSE50, KYSE220, and KYSE270) were analyzed using the GeneChip Human Genome U133 Plus 2.0 microarray (Affymetrix, Santa Clara, CA, USA), as described previously [32]. Genes with signal intensities of 250 or more were considered expressed genes.

Analysis of DNA Methylation

Analysis of DNA methylation was performed using an Infinium HumanMethylation450 BeadChip array, which covered 482,421 CpG and 3,091 non-CpG sites (Illumina, San Diego, CA), as described previously [33]. The methylation level of a CpG site was represented by a β value, which ranged from 0 (completely unmethylated) to 1 (completely methylated). A corrected β value was calculated using a measured beta value and the fraction of cancer cells in a sample [A corrected β value = the β value measured × 100/(the fraction of cancer cells in the sample (%))] [28].

All the CpG sites were grouped into 193,531 genomic "segments", each of which was defined by its location against a transcription start site (TSS) and relative location against a CGI. Genomic segments were divided into 299,563 genomic "blocks" smaller than 500 bp, and 292,265 genomic blocks on autosomes were used for the analysis. The numbers of genomic segments and blocks in this study were different from those in our previous study [30]. This is because genomic segments and blocks with probes that failed to produce reliable β value in any samples were excluded in the previous study [30], but not in this study. A DNA methylation level of a genomic block was evaluated using the mean of corrected β values of the CpG sites within the genomic block, and the methylation status of the genomic block was classified into unmethylated (corrected β value < 0.4), partially methylated ($0.4 \leq$ corrected β value < 0.8), and heavily methylated (corrected β value ≥ 0.8).

Selection of Genes of Cancer-Related Pathways

Six cancer-related pathways (the WNT pathway, the AKT/mTOR pathway, the MAPK pathway, the p53 pathway, cell cycle regulation, and mismatch repair) were selected because these pathways have been repeatedly reported to be altered in ESCCs [9–11,34–37]. Genes involved in the six cancer-related pathways were selected from the Kyoto Encyclopedia of Genes and Genomes Pathway Database (http://www.genome.jp/kegg/). Regarding the pathways activated in ESCCs, their negative regulators were selected. Regarding the pathways inactivated in ESCCs, their positive regulators and downstream effectors were selected. A total of 81 genes were selected for analyses of the six cancer-related pathways (Supplementary Table S4 and Figure S1). Among the 81 genes, 25 genes were analyzed for base substitutions, 67 genes for methylation silencing, and 11 genes for both (Supplementary Table S4 and Figure S1). For the analysis of methylation silencing, we used a CGI in a 200-bp upstream region from a transcription start site (TSS200) whenever probes were available in this region because DNA methylation of a CGI in a TSS200 is known to consistently silence its downstream gene [12,37–40] (Supplementary Table S5).

Western Blot Analysis

Proteins in total cell lysate (5 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and were transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). Phospho-p44/42 MAPK (ERK1/2) and p44/42 MAPK (ERK1/2) were detected using a rabbit polyclonal antibody against phospho-p44/42 MAPK (1:1,000; CST4370; Cell Signaling Technology, Danvers, MA), and a rabbit polyclonal antibody against p44/42 MAPK (1:1,000; CST4695; Cell Signaling Technology, Danvers, MA), respectively. Protein bands were visualized by enhanced chemiluminescence.

Clustering Analysis

Unsupervised hierarchical clustering analysis was performed using R 2.15 [R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/] with the Heatplus package [Alexander Ploner (2011) Heatplus: Heatmaps with row and/or column covariates and colored cluster, R package version 2.2.0] from Bioconductor [41]. The Euclidean distance was used as distance function both for samples and genes. From the 299,563 genomic blocks, 7,384 blocks located in TSS200 CGIs were used for the clustering analysis because methylation of TSS200 CGIs is known to silence their downstream genes [12,38,39].

Statistical Analysis

Overall survival (OS) was calculated from the date of diagnosis to the date of death or the final date of survival confirmation. Survival curves were drawn by the Kaplan–Meier method, and were compared by the log-rank test. Fisher's exact test was used to evaluate a significant difference in an association between a pathway alteration and clinicopathological characteristics. All statistical analyses were conducted by PASW statistics version 18.0.0 (SPSS Japan, Inc., Tokyo, Japan).

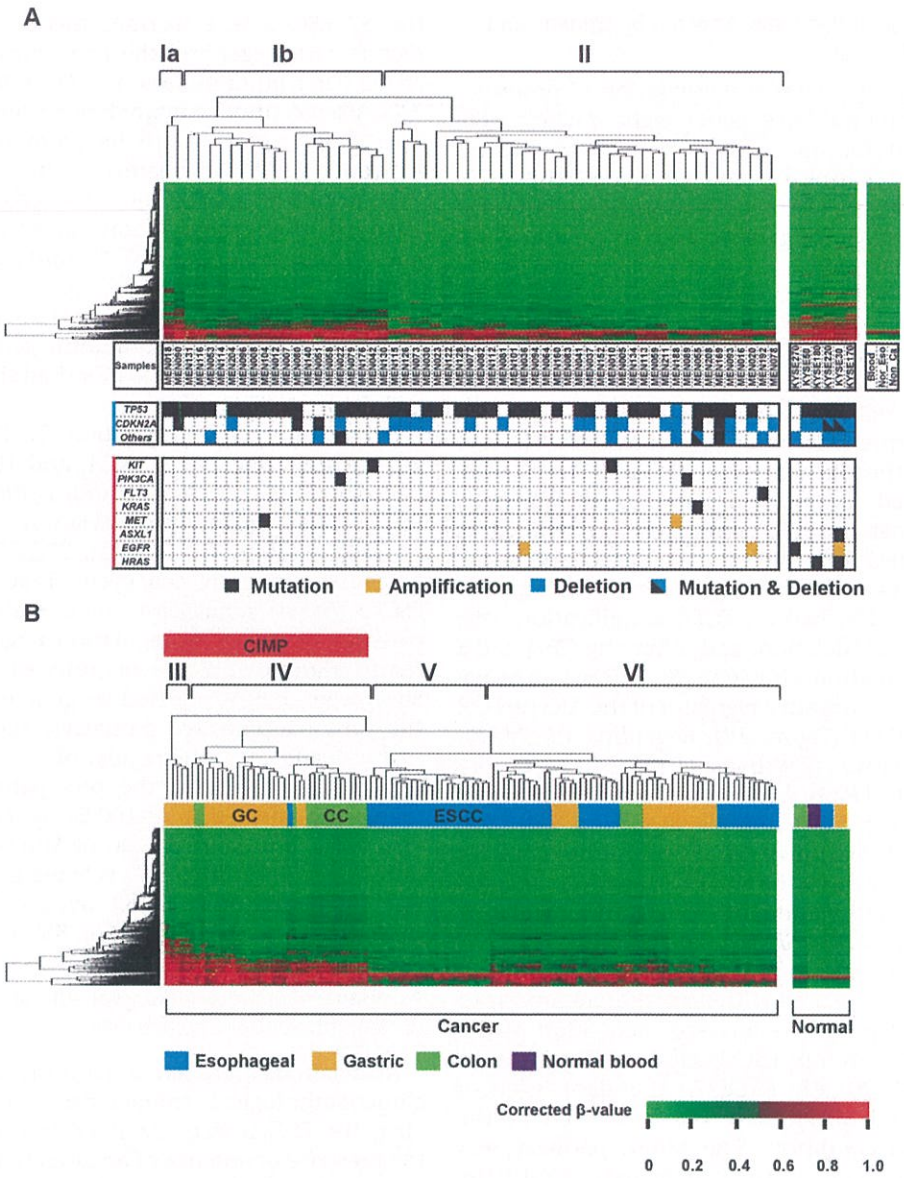

Figure 2. Associations between DNA methylation profile and gene mutations. (A) Unsupervised hierarchical clustering analysis using 7,384 genomic blocks with TSS200 CGIs. ESCCs in Clusters Ia and Ib had larger numbers of aberrantly methylated genes than those in Cluster II. Alterations of oncogenes and tumor suppressor genes were distributed in the three clusters with similar frequencies. (B) Unsupervised hierarchical clustering analysis of 120 cancers (57 ESCCs, 43 gastric cancers, and 20 colon cancers). Cancers in Clusters III and IV had larger numbers of aberrantly methylated genes than those in Clusters V and VI. Cancers in Cluster VI had larger numbers of aberrantly methylated genes than those in Cluster V, rather resembling those in Cluster IV.

We further compared the numbers of aberrantly methylated genes with those in gastric and colon cancers, in which the presence of the CIMP is known [30,42–46]. Unsupervised hierarchical clustering analysis was conducted using corrected methylation profiles of 120 cancers, including the 57 ESCCs, 43 gastric cancers [47] (Supplementary Figure S3A), and 20 colon cancers (NCBI GEO DataSets; Accession no. GSE42752) [48] (Supplementary Figure S3B). The analysis produced four major clusters (Figure 2B). Interestingly, cancers of the same tissues formed subclusters within the major clusters. In addition, gastric and colon cancers were split into Clusters IV and VI, which had a large and small, respectively, numbers of aberrantly methylated genes. ESCCs were in Clusters V and VI, both of which had a small number of aberrantly methylated genes, and cancers in clusters III and IV were considered CIMP positive. These results showed that the numbers of aberrantly methylated genes in ESCCs were smaller than those in gastric and colon cancers, and the CIMP is unlikely to be present in ESCCs.

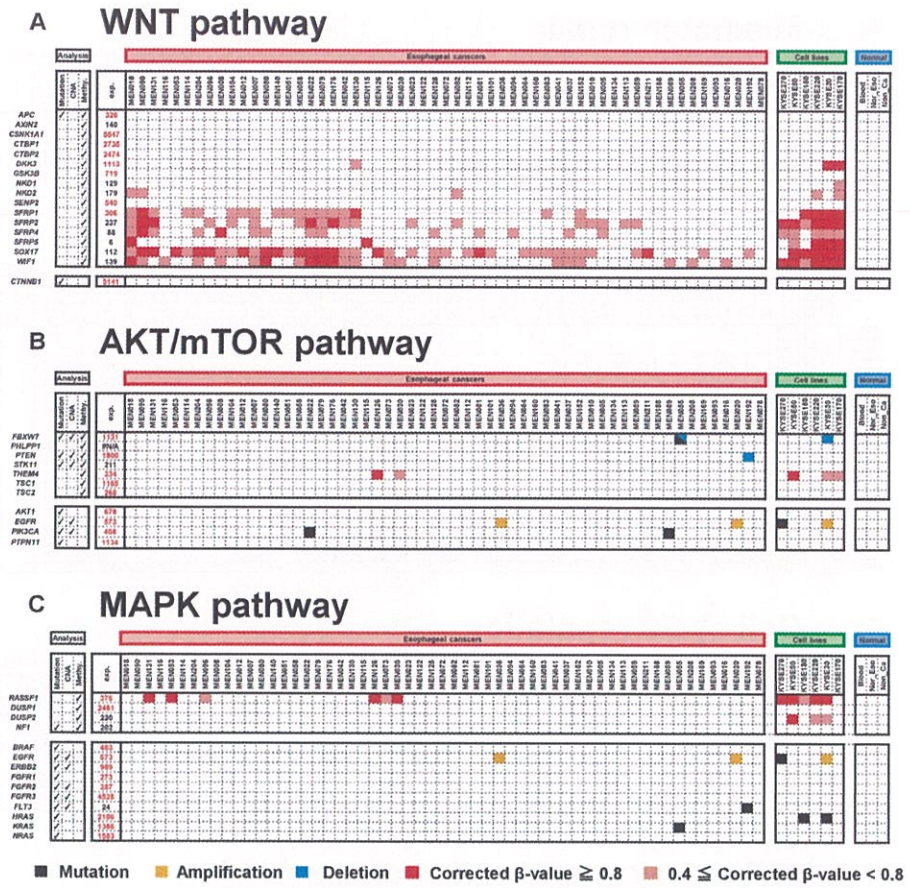

Figure 3. Genetic and epigenetic alterations in three growth-promoting pathways. (A) In the WNT pathway, none of the 57 ESCCs had an activating point mutation, but 19 ESCCs (33%) had heavy methylations of one or more of their 16 negative regulators. Expression levels obtained by microarray analysis of normal esophageal mucosae are also shown, and those higher than 250 are shown in red. Five of the six genes heavily methylated in ESCCs were not expressed in normal esophageal mucosae, suggesting that the five genes were methylated as passenger genes. (B) In the AKT/mTOR pathway, two ESCCs (4%) had activating mutations of *PIK3CA*, two ESCCs (4%) had *EGFR* amplification, and one ESCC (2%) had a *PTEN* deletion. Only one ESCC (2%) had heavy methylation of *THEM4*. (C) In the MAPK pathway, two ESCCs (4%) had an amplification of *EGFR*, four (7%) had heavy methylation of *RASSF1*, and two (4%) had point mutations of *FLT3* and *KRAS*.

DISCUSSION

A genome-wide methylation analysis and an extensive mutation analysis of 57 ESCCs were conducted here, and they showed (i) that 44 of the 57 ESCCs (77%) had 64 non-synonymous somatic mutations of 10 different potential driver genes (*ARID1A*, *CDKN2A*, *FBXW7*, *FLT3*, *KIT*, *KRAS*, *MET*, *MLH1*, *PIK3CA*, and *TP53*), (ii) that the number of aberrantly methylated genes in ESCCs was smaller than those in gastric and colon cancers, (iii) that ESCCs had at least one genetic or epigenetic alteration in the p53 pathway and cell cycle regulation with high frequencies (77% and 47%, respectively), and (iv) that 19 of the 57 ESCCs (33%) had heavy methylation of one gene or more of negative regulators in the WNT pathway. This is the first report in which genetic and epigenetic alterations were simultaneously analyzed in the same set of ESCC samples.

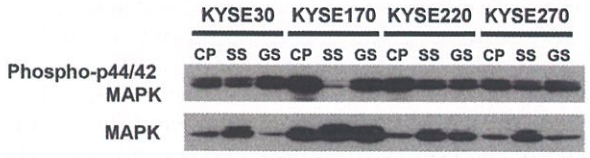

Figure 4. Activation of the MAPK pathway in ESCC cell lines. Phosphorylated MAPK and total MAPK levels were analyzed by Western blotting in four ESCC cell lines (KYSE30, KYSE170, KYSE220, and KYSE270) under conditions of cell proliferation (CP), serum-starvation (SS), and growth-stimulation (GS). In KYSE30, KYSE220, and KYSE270 abundant levels of phosphorylated MAPK were observed even in SS showing the MAPK pathway was constitutively activated. On the other hand, in KYSE170, only trace amounts of phosphorylated MAPK were observed in SS, showing the MAPK pathway was not activated.

Somatic mutations of six tumor-suppressor genes, four oncogenes, and CNVs of 10 genes were identified in this study. The most frequently mutated gene was *TP53* (70%), followed by *CDKN2A* (9%) and *PIK3CA* (4%) in this study, and the frequencies are close to the frequencies in the Catalogue of Somatic Mutations in

Table 1. Associations Between Pathways and Clinicopathological Findings

		Alterations in the WNT pathway			Alterations in the p53 pathway			Alterations in cell cycle regulation		
Category	Total number	Absent	Present	*P*-value*	Absent	Present	*P*-value*	Absent	Present	*P*-value*
Gender										
Male	51	33	18		11	40		25	26	
Female	6	5	1	0.652	2	4	0.610	3	3	1.000
Age										
<65	24	18	6		4	20		12	12	
≥65	33	20	13	0.394	9	24	0.524	16	17	1.000
Smoking										
Yes	51	33	18		10	41		24	27	
No	6	5	1	0.652	3	3	0.125	4	2	0.423
Drinking										
Yes	53	34	19		9	44		26	27	
No	4	4	0	0.290	4	0	**0.002**	2	2	1.000
Location										
Upper	9	7	6		2	7		7	2	
Middle	35	24	11		10	25		16	19	
Lower	13	7	2	0.468	1	12	0.309	5	8	0.156
T										
T1	12	6	6		3	9		5	7	
T2	8	6	2		1	7		6	2	
T3	36	25	11		9	27		16	20	
T4	1	1	0	0.498	0	1	0.823	1	0	0.288
N										
N0	10	6	4		2	8		6	4	
N1	47	32	15	0.717	11	36	1.000	22	25	0.504
M										
M0	43	31	12		9	34		24	19	
M1	14	7	7	0.192	4	10	0.715	4	10	0.123
Clinical stage										
IIA	8	5	3		2	6		5	3	
IIB	16	11	5		3	13		9	7	
III	19	15	4		4	15		10	9	
IVA/B	14	7	7	0.372	4	10	0.926	4	10	0.340

*P-values were calculated by Fisher's exact test.
Bold value is statistic significant (smaller than 0.05).

genome-wide methylation profiles obtained here will provide a valuable source for future data mining for novel tumor-suppressor genes and diagnostic targets in ESCCs.

Among the tumor-suppressive pathways, the p53 pathway was most frequently affected (44/57; 77%). *TP53* was inactivated by genetic alterations (40/57; 70%), and its downstream gene, *MIR34B*, was inactivated by epigenetic alterations (6/57; 11%). Regarding cell cycle regulation, *CDKN2A* was methylated or mutated with a relatively low frequency (7/57; 6%), but deletions were frequently observed (17/50; 30%). A cell cycle checkpoint gene, *CHFR*, was methylated in a small fraction (4/57; 7%). In total, 27 ESCCs (47%) had deregulation of the cell cycle. Taken together, the majority of ESCCs (50/57; 88%) had at least one alteration of genes involved in the p53 pathway or cell cycle regulation (Figure 5D), showing that these pathways are deeply involved in ESCC development.

Regarding the mismatch repair, two ESCC had genetic alterations of *MLH1*, whereas none of the 57 ESCCs had aberrant methylation of *MLH1*, showing that mismatch repair is not frequently inactivated in ESCCs. This was in contrast with previous studies that reported the presence of aberrant methylation of *MLH1* in 6–62% of ESCCs by methylation-specific PCR [17,37,53]. The study here employed quantitative methylation analysis that excludes overestimation of aberrant methylation and analysis of a critical region for transcriptional silencing [12], and the discrepancy with previous studies may be attributed to these differences in the methods.

Among the three growth-promoting pathways, genetic alterations were not frequent. However, the WNT pathway was considered to be activated by methylation of its negative regulators (*SFRP1*, *SFRP2*, *SFRP4*, *SFRP5*, *SOX17*, and *WIF1*) in 19 ESCCs (33%). The finding was in line with previous studies that reported an association between aberrant

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Supplemental Files

Supplemental file 1: Table S1: Clinical features of the 57 ESCC cases.

Supplemental file 2: Table S2: List of 55 cancer-related genes.

Supplemental file 3: Table S3: Primers used for dideoxy sequencing.

Supplemental file 4: Table S4: List of 81 genes of cancer-related pathways.

Supplemental file 5: Table S5: List of the positions of CpG sites.

Supplemental file 6: Table S6: Variations identified in the 57 ESCCs.

Supplemental file 7: Table S7: Relative reading depth of the 28 genes to the reference in the 57 ESCCs.

Supplemental file 8: Figure S1 The number of genes for the three kinds of analyses.

A total of 81 genes were selected for analyses of the six cancer-related pathways. Among the 81 genes, 25 genes were analyzed for base substitutions, 67 genes for methylation-silencing, and 11 genes for both. Among the 25 genes, 14 genes were analyzed for CNAs, and eight genes were analyzed for base substitutions, CNAs, and methylation-silencing.

Supplemental file 9: Figure S2 Detection of CNA by Ion PGM sequencer.

(A) Representative scattered plots of reading depth of the target regions between the ESCC and noncancerous mucosae. The slope shows RRDR. Red squares denote the eight target regions on *EGFR*. (B) Array-CGH data around the *EGFR* regions. Y-axis with boxes shows genomic locations. X-axis shows relative signal intensity (rSI) of the probes to the reference DNA. A number in the chart indicates an average of signal intensity of the probes in the target gene. (C) RRDR and rSI of *EGFR* and *CDKN2A* in three ESCCs (Round plots; RRDR, Square plots; rSI).

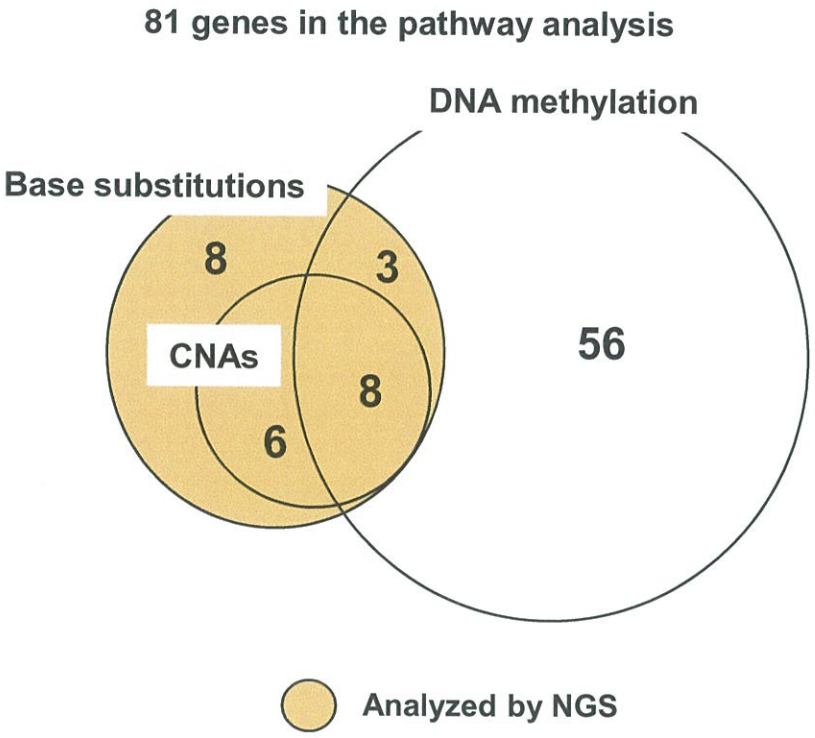

Figure S1
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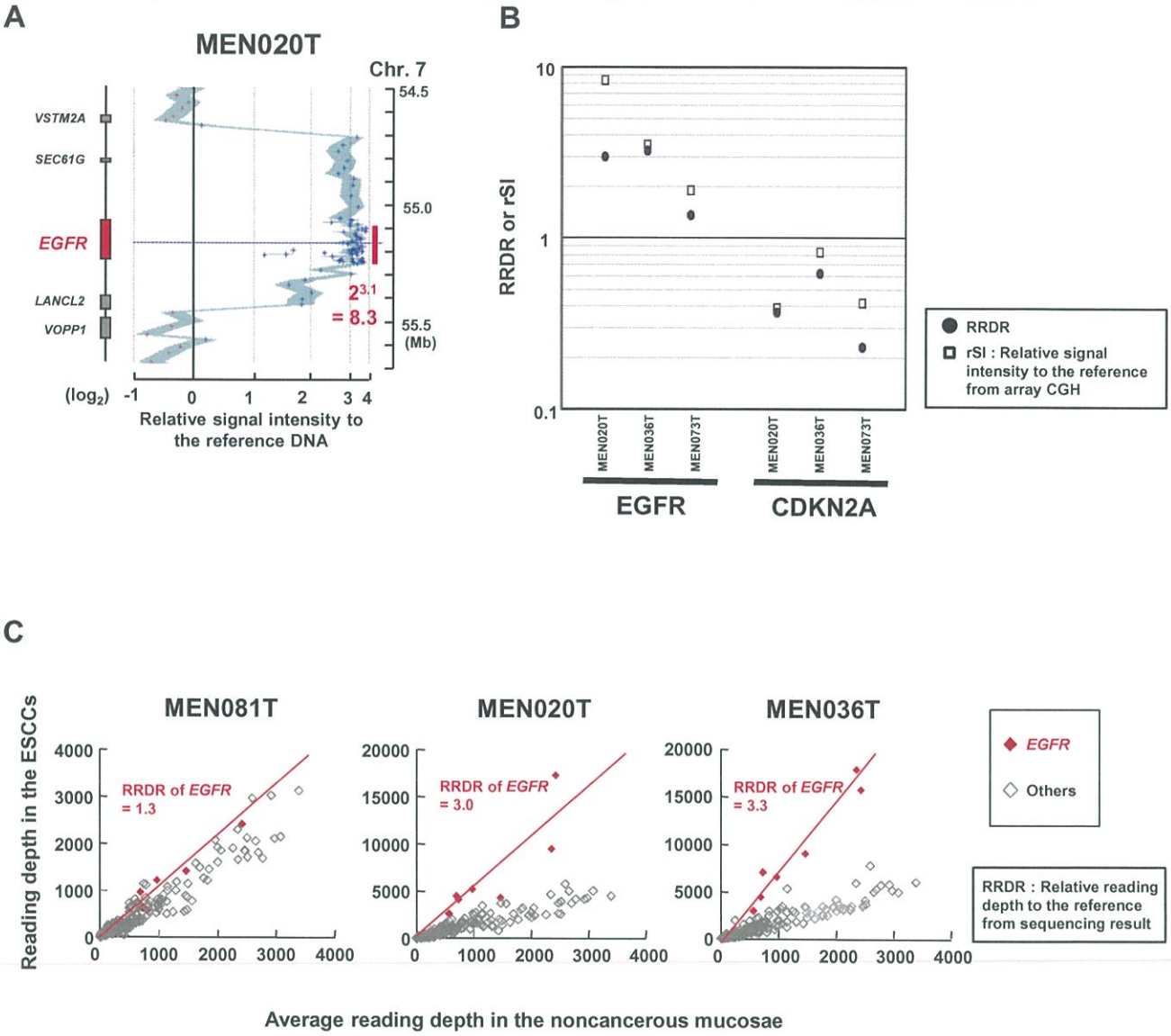

Figure S2
Kishino *et al.*

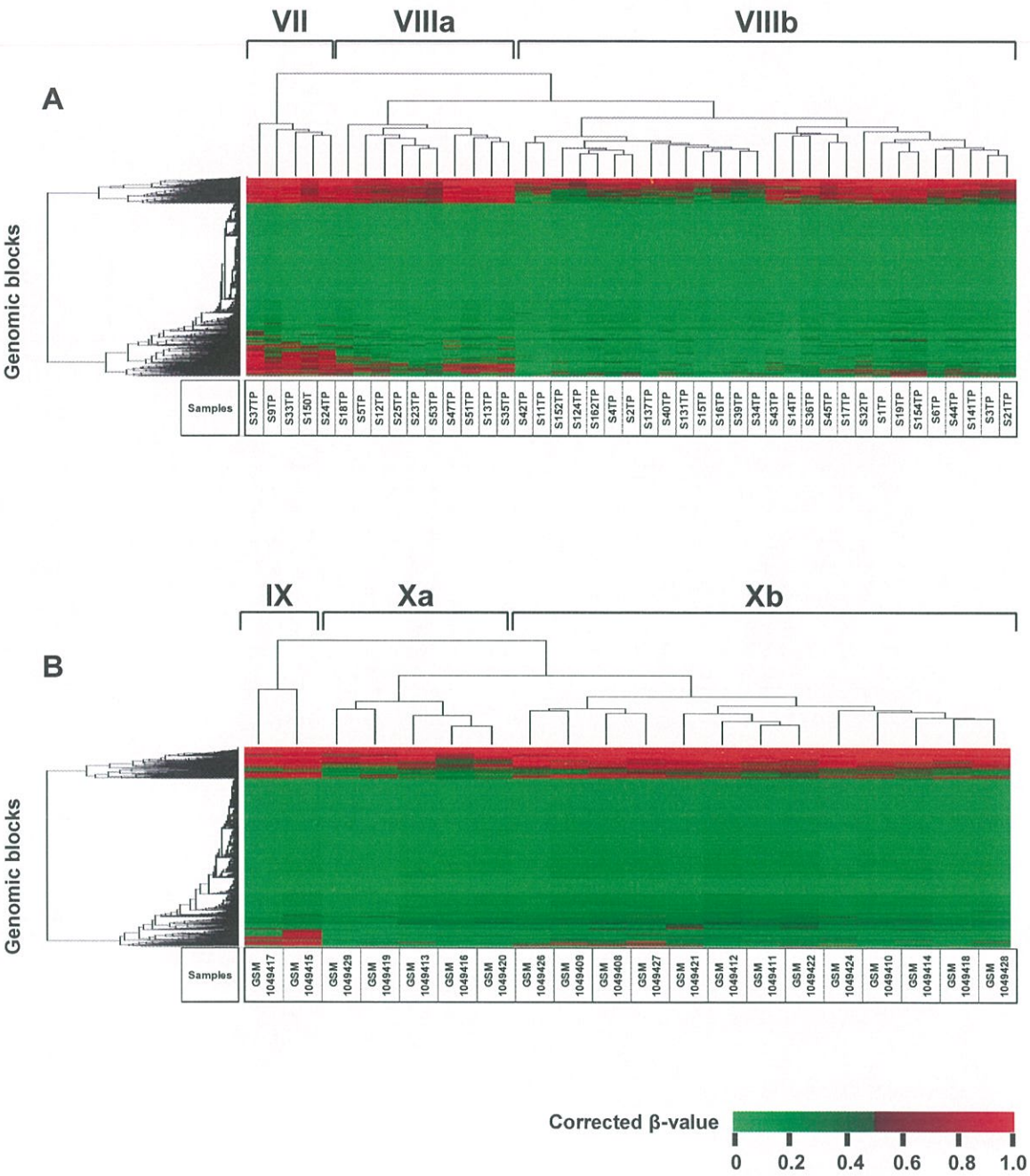

Figure S3
Kishino *et al.*

Table S1. Clinical features of the 57 ESCC cases

	Individual				Tumor (clinical diagnosis) UICC 6th				
Sample	Age	Gender	Smoking	Drinking	Location	T	N	M	cStage
MEN005	66	M	Yes	Yes	Mt	T3	N1	M0	III
MEN007	68	M	Yes	Yes	Lt	T1b	N1	M0	IIB
MEN008	61	M	Yes	Yes	Lt	T3	N1	M0	III
MEN010	74	M	Yes	Yes	Lt	T1b	N1	M0	IIB
MEN012	68	M	Yes	Yes	Lt	T1b	N0	M1b	IVB
MEN016	63	F	Yes	Yes	Mt	T3	N1	M0	III
MEN018	63	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN020	56	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN022	68	M	Yes	Yes	Mt	T1b	N1	M0	IIB
MEN023	61	M	Yes	Yes	Lt	T3	N1	M0	III
MEN030	49	M	Yes	Yes	Mt	T2	N1	M0	IIB
MEN036	63	M	Yes	Yes	Mt	T3	N1	M0	III
MEN037	64	M	Yes	Yes	Mt	T2	N0	M0	IIA
MEN041	65	M	Yes	Yes	Mt	T3	N1	M0	III
MEN042	70	M	Yes	Yes	Mt	T3	N1	M0	III
MEN051	75	M	Yes	No	Mt	T3	N1	M1b	IVB
MEN053	77	M	Yes	Yes	Mt	T2	N0	M0	IIA
MEN055	64	M	Yes	Yes	Ce	T3	N0	M1b	IVB
MEN058	58	M	Yes	Yes	Mt	T3	N0	M0	IIA
MEN059	67	M	Yes	Yes	Lt	T3	N0	M0	IIA
MEN064	66	M	Yes	Yes	Ce	T2	N1	M0	IIB
MEN072	61	F	Yes	Yes	Lt	T1b	N1	M0	IIB
MEN073	70	F	No	No	Mt	T3	N1	M0	III
MEN078	48	M	No	Yes	Mt	T1b	N1	M0	IIB
MEN079	69	M	Yes	Yes	Mt	T3	N0	M0	IIA
MEN080	67	M	Yes	Yes	Lt	T3	N1	M0	III
MEN081	66	M	Yes	Yes	Mt	T1b	N1	M1b	IVB
MEN082	67	M	Yes	Yes	Mt	T3	N1	M0	III
MEN083	75	M	Yes	Yes	Mt	T1b	N1	M0	IIB
MEN089	62	F	Yes	Yes	Mt	T3	N1	M0	III
MEN090	70	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN093	58	M	Yes	Yes	Mt	T3	N1	M0	III
MEN094	60	M	Yes	Yes	Mt	T3	N0	M0	IIA
MEN096	73	M	Yes	Yes	Lt	T3	N1	M0	III
MEN101	65	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN104	57	M	Yes	Yes	Ut	T4	N1	M0	III
MEN112	68	F	No	Yes	Mt	T3	N1	M0	III
MEN113	69	M	Yes	Yes	Ut	T3	N0	M0	IIA
MEN114	70	M	Yes	Yes	Lt	T3	N1	M0	III
MEN115	67	F	Yes	Yes	Mt	T1b	N1	M0	IIB
MEN116	64	M	Yes	Yes	Mt	T2	N1	M0	IIB
MEN122	69	M	No	No	Ut	T3	N0	M0	IIA
MEN126	69	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN128	70	M	Yes	Yes	Ut	T2	N1	M0	IIB
MEN130	74	M	Yes	Yes	Ut	T1b	N1	M0	IIB
MEN131	60	M	No	Yes	Ut	T2	N1	M0	IIB
MEN134	79	M	Yes	Yes	Lt	T3	N1	M0	III
MEN140	75	M	Yes	Yes	Ae	T3	N1	M1a	IVA
MEN152	55	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN160	64	M	Yes	Yes	Mt	T2	N1	M0	IIB
MEN169	79	M	Yes	Yes	Ut	T3	N1	M0	III
MEN176	54	M	Yes	Yes	Lt	T3	N1	M1b	IVB
MEN188	56	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN192	67	M	No	No	Mt	T1b	N1	M0	IIB
MEN204	65	M	Yes	Yes	Mt	T1b	N1	M0	IIB
MEN208	61	M	Yes	Yes	Mt	T3	N1	M1b	IVB
MEN211	55	M	Yes	Yes	Mt	T3	N1	M0	III

Table S2. List of 55 cancer-related genes.

Gene	Base substitution	CNA	Pathway
ABL1	✓	✓	
AKT1	✓		✓
ALK	✓		
APC	✓	✓	✓
ARID1A	✓		
ASXL1	✓	✓	
ATM	✓	✓	
BRAF	✓		✓
BRCA1	✓		
CDH1	✓	✓	
CDKN2A	✓	✓	✓
CSF1R	✓		
CTNNB1	✓		✓
EGFR	✓	✓	✓
EP300	✓		
ERBB2	✓	✓	✓
ERBB4	✓	✓	
FBXW7	✓	✓	✓
FGFR1	✓		✓
FGFR2	✓	✓	✓
FGFR3	✓	✓	✓
FLT3	✓	✓	✓
GNAS	✓		
H3F3A	✓		
HNF1A	✓		
HRAS	✓		✓
IDH1	✓		
JAK2	✓		
JAK3	✓		
KDR	✓	✓	
KIT	✓	✓	
KRAS	✓		✓
MET	✓	✓	
MLH1	✓	✓	✓
MLL3	✓	✓	
MPL	✓		
MSH2	✓		✓
MSH6	✓		✓
NF1	✓		✓
NOTCH1	✓		
NPM1	✓		
NRAS	✓		✓
PDGFRA	✓	✓	
PIK3CA	✓	✓	✓
PTEN	✓	✓	✓
PTPN11	✓		✓
RB1	✓	✓	✓
RET	✓	✓	
SMAD4	✓	✓	

Table S5. List of the positions of CpG sites.

ID of a block	Gene	Relation to a TSS	Relation to a CpG Island	Signaling pathway	# of CpG sites	Chromosome	Positions of probes
78446	*FBXW7*	TSS200	Island	Akt/mTOR	4	4	153456344, 153456325, 153456176, 153456369
258340	*PHLPP1*	TSS200	Island	Akt/mTOR	6	18	60382541, 60382543, 60382673, 60382585, 60382658, 60382639
160869	*PTEN*	TSS200	Island	Akt/mTOR	10	10	89623020, 89623157, 89623018, 89623122, 89623013, 89623071, 89623004, 89623076, 89623138, 89623142
18501	*THEM4*	TSS200	Island	Akt/mTOR	4	1	151882284, 151882157, 151882143, 151882287
260052	*STK11*	TSS200	Island	Akt/mTOR	3	19	1205696, 1205706, 1205715
151071	*TSC1*	5'UTR	Island	Akt/mTOR	1	9	135819856
227733	*TSC2*	TSS200	Island	Akt/mTOR	1	16	2097837
145721	*CDKN2A (p16)*	1stExon	Island	Cell cycle	1	9	21974704
199350	*CHFR*	TSS200	Island	Cell cycle	4	12	133464357, 133464323, 133464351, 133464327
103652	*CDKN1A*	TSS200	Island	Cell cycle	1	5	3664390
168878	*CDKN1C*	TSS200	Island	Cell cycle	1	11	2907008
145727	*CDKN2B*	TSS200	Island	Cell cycle	1	9	2209355
186649	*CDKN1B*	TSS200	Island	Cell cycle	2	12	12870119, 12870121
11646	*CDKN2C*	TSS200	Island	Cell cycle	3	1	51434293, 51434354, 51434335
263673	*CDKN2D*	TSS200	Island	Cell cycle	5	19	10679784, 10679729, 10679720, 10679775, 10679777
4967	*ZBTB17*	TSS200	Island	Cell cycle	1	1	16302665
56627	*RASSF1*	TSS200	Island	MAPK	7	3	50378529, 50378413, 50378407, 50378425, 50378527, 50378423, 50378431
94194	*DUSP1*	TSS200	Island	MAPK	1	5	172198326
38242	*DUSP2*	TSS200	Island	MAPK	1	2	96811264
244901	*NF1*	TSS200	Island	MAPK	7	17	29421884, 29421871, 29421804, 29421766, 29421851, 29421890, 29421832
54358	*MLH1*	TSS200	Island	Mismatch repair	2	3	37034814, 37034825
54363	*MLH1*	TSS200	Island	Mismatch repair	8	3	37035205, 37035228, 37035222, 37035200, 37035168, 37035158, 37035220, 37035207
34498	*MSH2*	TSS200	Island	Mismatch repair	4	2	47630251, 47630224, 47630147, 47630172
86775	*MSH3*	TSS200	Island	Mismatch repair	1	5	79950120
34551	*MSH6*	TSS200	Island	Mismatch repair	3	2	48010097, 48010117, 48010105
45463	*PMS1*	TSS200	Island	Mismatch repair	3	2	190648631, 190648634, 190648693
116765	*PMS2*	TSS200	Island	Mismatch repair	3	7	6048868, 6048832, 6048875
271621	*BAX*	TSS200	Island	p53	2	19	49458001, 49457927
80178	*CASP3*	TSS200	Island	p53	1	4	185570637
172789	*CD82*	TSS200	Island	p53	6	11	44586959, 44587138, 44587057, 44586968, 44587077, 44587044
103652	*CDKN1A*	TSS200	Island	p53	1	6	36646390
285595	*CHEK2*	TSS200	Island	p53	6	22	29137963, 29137905, 29137974, 29137992, 29137984, 29137908
118141	*CYCS*	TSS200	Island	p53	4	7	25164991, 25164974, 25165070, 25164994
173357	*DDB2*	TSS200	Island	p53	2	11	47236414, 47236405
183239	*EI24*	TSS200	Island	p53	4	11	125439225, 125439223, 125439275, 125439230
260892	*GADD45B*	TSS200	Island	p53	3	19	2476132, 2476129, 2476077
147500	*GADD45G*	TSS200	Island	p53	1	9	92219858
288368	*GTSE1*	TSS200	Island	p53	5	22	46692606, 46692562, 46692594, 46692631, 46692564
73534	*IGFBP7*	TSS200	Island	p53	4	4	57976561, 57976573, 57976566, 57976556
181066	*MIR34B*	TSS200	Island	p53	5	11	111383603, 111383576, 111383515, 111383624, 111383653
110508	*PERP*	TSS200	Island	p53	4	6	138428740, 138428736, 138428792, 138428833
258255	*PMAIP1*	TSS200	Island	p53	5	18	57567000, 57567149, 57567154, 57567158, 57567007
42917	*RPRM*	TSS200	Island	p53	5	2	154335386, 154335368, 154335379, 154335384, 154335348
30744	*RRM2*	TSS200	Island	p53	6	2	10262814, 10262820, 10262827, 10262822, 10262861, 10262787
108481	*SESN1*	TSS200	Island	p53	4	6	109416237, 109416235, 109416242, 109416245
7869	*SESN2*	TSS200	Island	p53	4	1	28585832, 28585863, 28585837, 28585917
232849	*SIAH1*	TSS200	Island	p53	2	16	48399813, 48399834
40448	*STEAP3*	TSS200	Island	p53	4	2	119981334, 119981253, 119981284, 119981187
218061	*THBS1*	TSS200	Island	p53	4	15	39873206, 39873260, 39873258, 39873209
134964	*TNFRSF10B*	TSS200	Island	p53	5	8	22926785, 22926898, 22926860, 22926862, 22926800
65741	*ZMAT3*	TSS200	Island	p53	3	3	178789707, 178789612, 178789599
88286	*APC*	TSS200	Island	WNT	8	5	112043137, 112043105, 112043080, 112043158, 112043117, 112043131, 112043188, 112043194
251203	*AXIN2*	TSS200	Island	WNT	1	17	63557887
92224	*CSNK1A1*	TSS200	Island	WNT	6	6	148931078, 148931071, 148931119, 148931087, 148931203, 148931083
68746	*CTBP1*	TSS200	Island	WNT	1	4	1243087
165340	*CTBP2*	TSS200	Island	WNT	5	10	126849762, 126849780, 126849704, 126849760, 126849624
170342	*DKK3*	TSS200	Island	WNT	6	11	12030268, 12030278, 12030375, 12030187, 12030272, 12030289
60841	*GSK3B*	TSS200	Island	WNT	3	3	119813301, 119813316, 119813310
233102	*NKD1*	TSS200	Island	WNT	2	16	50582193, 50582056
81362	*NKD2*	TSS200	Island	WNT	1	5	1009091
66546	*SENP2*	TSS200	Island	WNT	4	3	185303904, 185303898, 185303988, 185303959
136677	*SFRP1*	TSS200	Island	WNT	5	8	41167087, 41167113, 41167109, 41167107, 41166990
78637	*SFRP2*	TSS200	Island	WNT	7	4	154710353, 154710418, 154710371, 154710399, 154710421, 154710425, 154710373
119632	*SFRP4*	TSS200	Island	WNT	1	7	37956554
162008	*SFRP5*	TSS200	Island	WNT	6	10	99531765, 99531879, 99531934, 99531790, 99531797, 99531929
137633	*SOX17*	TSS200	Island	WNT	6	8	55370423, 55370429, 55370434, 55370334, 55370407, 55370336
191965	*WIF1*	TSS200	Island	WNT	2	12	65515290, 65515276

Among the genes involved in the AKT/mTOR, MAPK, WNT pathways, mismatch repair, cell cycle regulation, and the p53 pathway, genes with genomic blocks within TSS200 CGIs were selected from the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database.

Table S6. Variations identified in the 57 ESCCs.

Sample name	Gene	Chromosome	Region	Coverage	Frequency(%)	Coding region change	Amino acid change	Type	Zygosity
MEN007	*TP53*	chr17	7577120	2988	31.8	c.818G>T	p.Arg273Leu	SNV	Heterozygous
MEN008	*TP53*	chr17	7578271	360	30.3	c.578A>C	p.His193Pro	SNV	Heterozygous
MEN010	*TP53*	chr17	7577538	614	37.6	c.743G>A	p.Arg248Gln	SNV	Heterozygous
MEN010	*KIT*	chr4	55593461	191	42.4	c.1618G>C	p.Val540Leu	SNV	Heterozygous
MEN012	*TP53*	chr17	7578263	862	18.9	c.586C>T	p.Arg196*	SNV	Heterozygous
MEN018	*TP53*	chr17	7577573	1036	66.3	c.708C>A	p.Tyr236*	SNV	Heterozygous
MEN020	*TP53*	chr17	7577098	1883	27.1	c.840A>T	p.Arg280Ser	SNV	Heterozygous
MEN022	*TP53*	chr17	7578438^7578439	474	69.4	c.491_492insAA	p.Lys164fs	Insertion	Heterozygous
MEN022	*MLH1*	chr3	37067242	687	78.0	c.1153C>T	p.Arg385Cys	SNV	Heterozygous
MEN022	*PIK3CA*	chr3	178936082	923	35.4	c.1624G>A	p.Glu542Lys	SNV	Heterozygous
MEN023	*TP53*	chr17	7578190	695	56.4	c.659A>G	p.Tyr220Cys	SNV	Heterozygous
MEN030	*TP53*	chr17	7579358	79	64.6	c.329G>T	p.Arg110Leu	SNV	Heterozygous
MEN036	*TP53*	chr17	7577121	2169	30.3	c.817C>T	p.Arg273Cys	SNV	Heterozygous
MEN037	*TP53*	chr17	7577046	2556	69.5	c.892G>T	p.Glu298*	SNV	Heterozygous
MEN041	*TP53*	chr17	7577127	2308	34.4	c.811G>A	p.Glu271Lys	SNV	Heterozygous
MEN041	*TP53*	chr17	7578412	624	31.7	c.518T>A	p.Val173Glu	SNV	Heterozygous
MEN042	*TP53*	chr17	7577121	2202	13.6	c817C>T	p.Arg273Cys	SNV	Heterozygous
MEN042	*TP53*	chr17	7577124	1907	18.2	c.814G>A	p.Val272Met	SNV	Heterozygous
MEN042	*KIT*	chr4	55593461	175	41.1	c.1618G>C	p.Val540Leu	SNV	Heterozygous
MEN051	*CDKN2A*	chr9	21971159	170	37.6	c.242G>C	p.Arg81Pro	SNV	Heterozygous
MEN053	*TP53*	chr17	7579358	118	39.0	c.329G>T	p.Arg110Leu	SNV	Heterozygous
MEN055	*KRAS*	chr12	25378562	3482	28.4	c.436G>A	p.Ala146Thr	SNV	Heterozygous
MEN055	*TP53*	chr17	7577546..7577547	378	63.2	c.734_735delGC	p.Gly245fs	Deletion	Heterozygous
MEN055	*FBXW7*	chr4	153259008	64	65.6	c.807delG	p.Met269fs	Deletion	Heterozygous
MEN055	*CDKN2A*	chr9	21971170^21971171	616	67.0	c.230_231insCCTGC	p.Ala77fs	Insertion	Heterozygous
MEN059	*TP53*	chr17	7578443	262	47.3	c.487T>A	p.Tyr163Asn	SNV	Heterozygous
MEN072	*TP53*	chr17	7578190	372	79.0	c.659A>G	p.Tyr220Cys	SNV	Heterozygous
MEN079	*TP53*	chr17	7577579	297	47.5	c.702C>A	p.Tyr234*	SNV	Heterozygous
MEN080	*TP53*	chr17	7577124	395	23.0	c.814G>A	p.Val272Met	SNV	Heterozygous
MEN080	*TP53*	chr17	7578264^7578265	210	30.0	c.584_585insT	p.Ile195fs	Insertion	Heterozygous
MEN080	*CDKN2A*	chr9	21971186	98	52.0	c.215C>T	p.Pro72Leu	SNV	Heterozygous
MEN082	*TP53*	chr17	7578445	305	25.2	c.485T>G	p.Ile162Ser	SNV	Heterozygous
MEN089	*PIK3CA*	chr3	178936082	685	22.5	c.1624G>A	p.Glu542Lys	SNV	Heterozygous
MEN090	*TP53*	chr17	7577538	534	19.9	c.743G>A	p.Arg248Gln	SNV	Heterozygous
MEN090	*CDKN2A*	chr9	21974742	201	35.8	c.85C>G	p.Arg29Gly	SNV	Heterozygous
MEN090	*CDKN2A*	chr9	21974744	201	35.8	c.83T>C	p.Val28Ala	SNV	Heterozygous
MEN093	*TP53*	chr17	7574003	940	41.8	c.1024C>T	p.Arg342*	SNV	Heterozygous
MEN094	*TP53*	chr17	7578271	547	17.0	c.578A>T	p.His193Leu	SNV	Heterozygous
MEN096	*TP53*	chr17	7577547	661	31.8	c.734G>A	:p.Gly245Asp	SNV	Heterozygous
MEN101	*TP53*	chr17	7578190	212	38.7	c.659A>G	p.Tyr220Cys	SNV	Heterozygous
MEN104	*TP53*	chr17	7578235	521	22.5	c.614A>G	p.Tyr205Cys	SNV	Heterozygous
MEN104	*MET*	chr7	116339673	682	15.8	c.535G>A	p.Ala179Thr	SNV	Heterozygous
MEN112	*TP53*	chr17	7577094	2227	28.5	c.844C>T	p.Arg282Trp	SNV	Heterozygous
MEN112	*TP53*	chr17	7578239	475	32.8	c.610G>T	p.Glu204*	SNV	Heterozygous
MEN113	*TP53*	chr17	7577130	1109	50.8	c.808T>A	p.Phe270Ile	SNV	Heterozygous
MEN113	*TP53*	chr17	7577577	637	34.7	c.704delA	p.Asn235fs	Deletion	Heterozygous
MEN115	*TP53*	chr17	7578190	541	67.5	c.659A>G	p.Tyr220Cys	SNV	Heterozygous
MEN116	*TP53*	chr17	7577558	689	50.2	c.723delC	p.Ser241fs	Deletion	Heterozygous
MEN126	*TP53*	chr17	7578479	297	87.9	c.451C>T	p.Pro151Ser	SNV	Heterozygous
MEN128	*TP53*	chr17	7577538	1052	54.6	c.743G>A	p.Arg248Gln	SNV	Heterozygous
MEN130	*TP53*	chr17	7578406	266	25.6	c.524G>A	p.Arg175His	SNV	Heterozygous
MEN131	*TP53*	chr17	7578413	346	63.3	c.517G>T	p.Val173Leu	SNV	Heterozygous
MEN134	*TP53*	chr17	7578478	148	77.0	c.452C>G	p.Pro151Arg	SNV	Heterozygous
MEN160	*TP53*	chr17	7579355	262	40.8	c.332T>A	p.Leu111Gln	SNV	Heterozygous
MEN169	*ARID1A*	chr1	27088742	113	61.9	c.2351G>A	p.Gly784Asp	SNV	Heterozygous
MEN169	*TP53*	chr17	7577076..7577077	704	69.6	c.861_862delGA	p.Glu287fs	Deletion	Heterozygous
MEN169	*CDKN2A*	chr9	21970984	216	59.7	c.374A>G	p.Asp125Gly	SNV	Heterozygous
MEN169	*CDKN2A*	chr9	21970987	215	60.5	c.371G>A	p.Arg124His	SNV	Heterozygous
MEN169	*CDKN2A*	chr9	21970990	183	56.8	c.368A>G	p.His123Gly	SNV	Heterozygous
MEN169	*CDKN2A*	chr9	21970991	183	56.8	c.367C>G	p.His123Gly	SNV	Heterozygous
MEN176	*TP53*	chr17	7578529	744	11.3	c.401T>C	p.Phe134Ser	SNV	Heterozygous
MEN192	*FLT3*	chr13	28592610	417	23.7	c.2535G>C	p.Arg845Ser	SNV	Heterozygous
MEN204	*TP53*	chr17	7578437	157	67.5	c.493C>T	p.Gln165*	SNV	Heterozygous
MEN208	*TP53*	chr17	7578394	169	81.7	c.536A>T	p.His179Leu	SNV	Heterozygous
KYSE30	*HRAS*	chr11	533874	719	70.1	c.182A>T	p.Gln61Leu	SNV	Heterozygous
KYSE30	*TP53*	chr17	7579358	332	24.1	c.329G>T	p.Arg110Leu	SNV	Heterozygous
KYSE30	*ASXL1*	chr20	31022641	904	32.2	c.2126C>T	p.Ala709Val	SNV	Heterozygous
KYSE30	*CDKN2A*	chr9	21971000	117	99.1	c.358G>T	p.Glu120*	SNV	Homozygous
KYSE50	*TP53*	chr17	7579386	334	100.0	c.301A>T	p.Lys101*	SNV	Homozygous
KYSE170	*TP53*	chr17	7577094	740	20.3	c.448C>G	p.Arg150Gly	SNV	Heterozygous
KYSE180	*HRAS*	chr11	534289	278	10.4	c.34G>A	p.Gly12Ser	SNV	Heterozygous
KYSE180	*TP53*	chr17	7578265	759	99.9	c.188T>C	p.Ile63Thr	SNV	Homozygous
KYSE220	*TP53*	chr17	7577539	563	98.0	c.346C>T	p.Arg116Trp	SNV	Homozygous
KYSE220	*CDKN2A*	chr9	21971153	179	98.3	c.205G>T	p.Glu69*	SNV	Homozygous
KYSE270	*EGFR*	chr7	55259524	1407	26.7	c.2582T>A	p.Leu861Gln	SNV	Heterozygous

Table S7. Relative reading depth of the 28 genes to the reference in the 57 ESCCs.

Gene	The number of amplicons	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ERBB4	8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MLH1	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
PIK3CA	7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FGFR3	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
PDGFRA	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
KIT	9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
KDR	9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FBXW7	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
APC	7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
EGFR	8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MET	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SMO	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MLL3	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CDKN2A	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ABL1	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
RET	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
PTEN	7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FGFR2	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ATM	17	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FLT3	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
RB1	8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CDH1	7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TP53	8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ERBB2	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SMAD4	8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
STK11	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AKT1	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SMARCB1	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

A gene in a sample with potential amplification is marked in orange, and a gene in a sample with potential deletion is marked in bl[illegible]