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Low-grade serous carcinomas of the ovary contain very few point mutations

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Abstract

It has been well established that ovarian low-grade and high-grade serous carcinomas are fundamentally different types of tumours. While the molecular genetic features of ovarian high-grade serous carcinomas are now well known, the pathogenesis of low-grade serous carcinomas, apart from the recognition of frequent somatic mutations involving *KRAS* and *BRAF*, is largely unknown. In order to comprehensively analyse somatic mutations in low-grade serous carcinomas, we applied exome sequencing to the DNA of eight samples of affinity-purified, low-grade, serous carcinomas. A remarkably small number of mutations were identified in seven of these tumours: a total of 70 somatic mutations in 64 genes. The eighth case displayed mixed serous and endometrioid features and a mutator phenotype with 783 somatic mutations, including a nonsense mutation in the mismatch repair gene, *MSH2*. We validated representative mutations in an additional nine low-grade serous carcinomas and 10 serous borderline tumours, the precursors of ovarian low-grade, serous carcinomas. Overall, the genes showing the most frequent mutations were *BRAF* and *KRAS*, occurring in 10 (38%) and 5 (19%) of 27 low-grade tumours, respectively. Except for a single case with a *PIK3CA* mutation, other mutations identified in the discovery set were not detected in the validation set of specimens. Our mutational analysis demonstrates that point mutations are much less common in low-grade serous tumours of the ovary than in other adult tumours, a finding with interesting scientific and clinical implications.

Keywords

ovarian cancer; exome sequencing; BRAF; KRAS; somatic mutations

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Author contributions

SJ, KWK, VEV, BV and NP designed the study and analysed sequencing data; T-LW, RJK, KN and I-MS designed the study, collected and purified samples, performed clinical correlation and prepared the manuscript.

Introduction

Ovarian cancer is not the most common gynaecological malignancy but it is the most lethal, accounting for nearly 140 200 deaths worldwide [1]. It is a heterogeneous disease composed of several distinctly different cell types. Among them, low-grade serous carcinoma (LGSC) constitutes a relatively unusual but distinctive type that tends to affect younger women more than high-grade serous carcinoma, the most common histopathological type [2–5]. In the past, it was thought that LGSCs progressed to high-grade serous carcinomas but it is now recognized that LGSCs and high-grade serous carcinomas generally develop along distinctly different molecular pathways [6]. As compared to high-grade ovarian serous carcinomas, LGSCs proliferate slowly and are clinically less aggressive. Although LGSCs have been termed 'low-grade', patients with this disease not infrequently develop recurrences and experience a protracted clinical course, requiring multiple surgical interventions, before succumbing to the disease, and the 10 year survival is < 50% [7].

LGSCs may develop from serous cystadenomas, which can progress to a serous borderline tumour (SBT), also known as atypical proliferative serous tumours (APSTs), non-invasive (micropapillary) LGSCs, then to invasive LGSC [8–11]. Hemizygous ch1p36 deletion and ch9p21 homozygous or hemizygous deletions may play an important role in this pathway, because deletions of both chromosomal regions are more frequent in LGSCs than in SBTs (APSTs) [12]. Mutational analyses of candidate genes have shown that both LGSC (non-invasive and invasive) and SBTs (APSTs) harbour somatic mutations in *KRAS* and *BRAF* in approximately half of the cases [13–16]. The aim of the present study was to comprehensively analyse the mutation profile of ovarian LGSCs by exome sequencing. Genome-wide mutation profiles of ovarian high-grade serous carcinomas [17] and clear cell carcinomas have recently been reported [18, 19]. The results reported here complement the other studies and highlight genetic differences among the different types of ovarian carcinomas.

Materials and methods

Tissue specimens

We analysed the exomes of eight LGSCs in a 'discovery set' and all specimens were reviewed by two gynaecological pathologists (RJK and IMS), using the criteria previously detailed [33], and microscopically all tumours exhibited a low-grade nuclear feature. These consisted of six carcinomas exhibiting morphologically pure serous differentiation and two with serous plus either clear cell or endometrioid features, respectively. Among them, four cases were recurrent and four were primary tumours. Tumour cells from LGSCs were isolated from fresh surgical specimens by collagenase I digestion, followed by epithelial cell enrichment using Dynal beads coated with Epi-CAM antibodies, as previously described [18]. The affinity-purified tumour cells were cultured in glass chamber slides overnight, fixed using 3% paraformaldehyde at room temperature for 10 min, and washed three times in phosphate-buffered solution containing 0.2% Triton X-100 (Sigma). The cells were then incubated with an antibody reacting to cytokeratin 17 (DAKO, Carpentaria, CA, USA) at a dilution of 1 : 20 at room temperature for 1 h. The immunoreactivity was visualized using the DAKO EnVision (HRP) + system (DAKO), following the manufacturer's protocol. Haematoxylin was used as a nuclear counterstain. To determine the purity of tumour cells after isolation, we determined the percentage of positive cells, as defined by intense nuclear staining, by randomly counting at least 100 cells at ×20 magnification.

Genomic DNA preparation

Genomic DNA (from both tumour and normal tissues) was purified using Qiagen DNA blood kits, following the manufacturer's protocol. The DNA from the normal counterpart came from peripheral blood lymphocytes in three cases, from stromal cells of normal fallopian tube in four cases and from normal liver in one case. In the validation set, there were nine LGSCs (four recurrent and five primary tumours) and 11 SBTs (APSTs), the precursor of LGSCs. The criteria used to select SBT cases were based on the following morphological features: extensive epithelial stratification; tufting; and detachment of individual cells and small cell clusters besides hierarchical branching, with successively smaller papillae emanating from the larger, more centrally located papillae. Of note, to warrant a diagnosis of SBT, we included only those cases with stratification and budding in at least 10% of the tumour. Among the 11 SBTs (APSTs), there were two containing a noninvasive (*in situ*) LGSC component. To purify genomic DNA from SBTs (APSTs), we incubated fresh tissue fragments of SBTs (APSTs) with 0.5% trypsin and EDTA at 37 °C for 20 min with agitation. The tumour cells on the surface of papillae were gently scraped off, the epithelial cells were seeded in tissue culture flasks overnight and blood cells were removed after several washes before the attached epithelial cells were harvested for DNA purification, as described above. Tissue acquisition was approved by the Institutional Research Board.

Exome sequencing and analysis

Preparation of Illumina genomic DNA libraries and exome and targeted subgenomic DNA capture were performed as described [18]. Briefly, after capture of the coding sequences of the targeted genes using a SureSelect Enrichment System, the DNA was sequenced using an Illumina GAIIx Genome Analyser. Paired-end sequencing, resulting in 75 bases from each end of the fragments, was employed. The tags were aligned to the human genome reference sequence (hg18) using the Eland algorithm of CASAVA 1.6 software (Illumina). The chastity filter of the Base-Call software of Illumina was used to select sequence reads for subsequent analysis. The ELANDv2 algorithm of CASAVA 1.6 software (Illumina) was then applied to identify point mutations and small insertions and deletions. Known polymorphisms recorded in dbSNP were removed from the analysis. A mismatched base was considered to be a putative somatic mutation only when: (a) it had a QS score > 20; (b) it was identified in more than three distinct tags; (c) the percentage of mutant tags in the tumour was > 10%; (d) it was not found in > 2% of the tags in the matched normal sample; (e) it was a non-synonymous change; (f) there were no repeated regions homologous to the mutated sequence (as determined by BLAT analysis); and (g) the normal distinct Phred coverage was > 10.

Sanger sequencing for validation

Putative mutations identified by exome sequencing were validated by Sanger sequencing. Those that were detected through Sanger sequencing were considered true somatic mutations. Additional LGSCs and SBTs (APSTs) were sequenced using conventional Sanger methods to identify the prevalence of mutations in *KRAS, BRAF, PIK3CA, ARID1A, TSPAN11, STYK1, RNF214, DDC* and *SMARCA4*. PCR and Sanger sequencing were performed as previously described [18, 20]. To confirm mutations based on exome sequencing data, we used the primers specific for the exon the potential mutation was found. To analyse the mutation status of the candidate genes in additional cases, we screened the entire coding region.

Results

To maximize sensitivity for detecting somatic mutations, we analysed neoplastic cells enriched by immunopurification from fresh specimens. Immunostaining of the purified epithelial cells confirmed that > 90% of them were epithelial. The cells isolated from eight patients were used to determine the sequences of the approximately 18 000 protein-encoding genes listed in the RefSeq database (see Supporting information, Table S1). DNA from the normal tissues of each of these eight patients was also used for exomic sequencing. Using the Illumina GAIIx platform, the average coverage of each nucleotide in the targeted regions was 75-fold and 92.1% of those bases were represented in at least 10 reads.

OV207 displayed more than 50-fold more mutations than the other tumours and this tumour was found to harbour a somatic nonsense mutation in *MSH2* (g.chr2 : 474969666C > T; c. 970C > T; p.324Q > X). Table S2 (see Supporting information) lists the 783 genes with somatic mutations found in OV207. Accordingly, OV207 was considered to be mismatch-repair deficient and was not considered for further analysis. Using stringent criteria for analysing the data from the other seven tumours [18], we were able to detect 85 somatic mutations (all listed in Table 1) and, among them, 70 somatic mutations in 65 genes could be confirmed by Sanger sequencing, and thus the false-positive rate of exome sequencing in this study was 17.6%. The validated somatic mutations per tumour averaged 10 for the 7 cases (range 0–24; Table 1). The six morphologically pure LGSCs (OV202, OV203, OV204, OV205, 0V206 and OV209) harboured ≤20 mutations, and one low-grade serous tumour (OV208) showing focal clear cell feature had 24 mutations. Thus, the somatic non-synonymous and splice site mutations/tumour was 7.5 in the 6 morphologically pure LGSCs. OV207, which was presumably mismatch-repair-deficient, exhibited mixed serous and endometrioid features. One tumour, OV202, did not harbour any detectable point mutations and its clinico-pathological features were similar to those of other low-grade serous neoplasms. The mutations detected in the pure LGSCs included *BRAF, KRAS, STYK1, TSPAN11, RNF214, CCDC76* and *SPATA5*.

Using Sanger sequencing, we determined the mutation status of *KRAS, BRAF, PIK3CA, ARID1A, TSPAN11, STYK1, RNF214, DDC* and *SMARCA4* in affinity-purified tumour samples from additional cases, including nine LGSCs and 10 SBTs (APSTs). *PIK3CA, ARID1A, TSPAN11, RNF214* and *DDC* were selected for further study, based on their mutations being found in more than one case. *KRAS* and *BRAF* were analysed because mutations in both genes have been reported in LGSCs. *SMARCA4* was selected, despite being mutated in only the sample OV207, because it encodes Brg1, a component of the SWI/SNF chromatin remodelling complex, by interacting with ARID1A. *STYK1* was also studied because it encodes a receptor protein tyrosine kinase that may be of biological and translational significance. We found that besides *BRAF, KRAS* and *PIK3CA*none of the other genes showed somatic mutations in any of the samples in the validation set (Table 2).

Combining specimens from both the discovery and validation sets revealed that among 15 morphologically pure LGSCs, four contained *KRAS* mutations, three contained *BRAF* mutations and one had a *PIK3CA* mutation. As in previous reports [6, 13, 21], mutations in *KRAS* and *BRAF* in those cases were mutually exclusive, therefore seven (47%) of 15 low-grade serous carcinomas harboured either *KRAS* or *BRAF* mutations. Table 2 summarizes the mutational profiles and clinical features in all the cases. The histopathological appearance and clinical outcome appeared indistinguishable between low-grade tumours with mutations in *KRAS* and *BRAF* and those without, although larger studies are required to confirm this observation. In contrast to a recent study [22] reporting rare *BRAF* mutations in advanced-stage LGCSs, we observed a significantly higher frequency of *BRAF* mutations which occurred in three of 15 (20%, 95% CI 0–40%) advanced-stage LGSCs, and one of

these patients died of the disease 42 months after diagnosis (Table 1, Figure 1). In SBTs (APSTs), a *KRAS* mutation was found in one case and *BRAF* mutations were detected in seven cases. We did not identify mutations in *PIK3CA, ARID1A, TSPAN11, STYK1, RNF214, DDC* or *SMARCA4* in SBTs (APSTs).

Discussion

Our exome sequencing confirms previous findings that ovarian LGSCs frequently harbour somatic activating mutations in *BRAF* and *KRAS* [13–16]. Although rare mutations can be detected in other coding sequences, our data indicate that mutations of *BRAF* and *KRAS* represent the most common molecular genetic changes in these tumours and that constitutive activation in the KRAS–BRAF–MEK–MAPK pathway resulting from these mutations plays a pivotal role in their pathogenesis. In fact, active MAPK is more frequently observed in low-grade serous tumours than in high-grade ovarian serous carcinomas, which rarely have mutations in either *BRAF* or *KRAS* [23]. This observation suggests that inhibitors of BRAF and MEK could potentially be used to treat patients with advanced-stage LGSCs. Of note, it has been reported that *BRAF* and *KRAS* mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancers [21, 24]. This is important because, unlike high-grade serous carcinoma, LGSCs are notorious for their poor response to conventional platinum-based chemotherapeutic regimens. An ongoing phase II trial (GOG 0239) of AZD6244, a MEK inhibitor, should determine whether there is clinical benefit in targeting MEK pathway for patients with LGSC and whether the response is associated with mutations of *BRAF* and *KRAS*.

The findings from the current study also highlight the distinctive molecular genetic features of LGSC as compared to other histological subtypes of ovarian cancer. It has previously been proposed that ovarian surface epithelial tumours can be broadly classified into type I and type II tumours [2, 8]. Type I tumours include LGSCs, low-grade endometrioid, clear cell and mucinous carcinomas, and type II tumours are composed, for the most part, of high-grade serous carcinomas. Fundamental differences between the molecular genetic features of type I and type II tumours have been identified in several reports. Those studies concluded that the type I tumours are relatively genetically stable and contain somatic mutations of *KRAS, BRAF, PTEN, PIK3CA CTNNB1, ARID1A* and *PPP2R1A* but rarely *TP53*. In contrast, type II tumours are chromosomally unstable and harbour *TP53* mutations in > 95% of cases; they rarely have the mutations found in the type I tumours [17, 18, 20, 25]. Thus, the exome sequencing of LGSC as reported in this study, together with our previous exome sequencing study in ovarian clear cell carcinoma, provides cogent evidence that the type I tumours are highly heterogeneous and are characterized by a distinct repertoire of mutated genes.

Occasionally, LGSCs appear to contain histopathological elements characteristic of other type I tumours, such as endometrioid or clear cell carcinomas. These ‘mixed’ tumours present diagnostic and managerial challenges because their behaviour and molecular features are not well understood. Our discovery set contained two such tumours. Mutational analysis of these two cases provided evidence that these histologically mixed tumours molecularly resemble endometrioid and clear cell carcinoma more than LGSC, because they harboured *PIK3CA* or *ARID1A* mutations, which are found in 30% of endometrioid and 50% of clear cell carcinomas, respectively [18–20, 26, 27] (Table 2). Moreover, OV207, which showed endometrioid features, was characterized by a mutator phenotype associated with a mutation in a mismatch-repair gene. Such mutator phenotypes are commonly found in endometrioid carcinomas. As not all LGSCs have mutations in *KRAS* or *BRAF*it is possible that the tumour-promoting functions of *KRAS*- and *BRAF*-mutated genes are conferred by other mutations or epigenetic activation in the same MEK pathway [28]. To

this end, a previous immunohistochemistry study has shown that active (phosphorylated) MAPK is present not only in all low-grade serous tumours with either *KRAS* or *BRAF* mutation but also in 41% of tumours without such mutations [23].

Perhaps the most interesting aspect of our study was the rarity of point mutations. On average, morphologically pure LGSCs averaged 7.5 somatic non-synonymous and splice site mutations/tumour. This number is far lower than in most common tumours of adults, in which 25–75 somatic mutations/tumour are commonly observed. It is lower than in the three other types of gynaecological cancers studied (34 mutations/tumour in ovarian clear cell carcinoma [18], 48 mutations/tumour in high-grade serous carcinoma [17], 45 mutations/tumour in uterine serous carcinoma), and even lower than in paediatric tumours, such as medulloblastomas [29]. Two independent conclusions can be made from this observation. First, the precursor cells for LGSC must have not replicated much prior to the initiation of tumourigenesis. Second, there must have been relatively few bottlenecks once this initiation occurred. If either of these conclusions were invalid, then a much larger number of mutations— mostly passengers—would have been observed [30]. A corollary of this conclusion is that the ratio of driver gene mutations (those which confer a selective growth advantage) to passenger gene mutations in LGSCs should be higher than is usually observed in other adult tumour types. It will be interesting to see whether the genes mutated at low frequency in LGSCs turn out to be drivers after further genetic and functional analyses.

Our data suggest that LGSCs do not require very many mutational 'hits' to achieve malignancy [31]. It is thereby possible that targeted therapeutic agents, such as those active against BRAF [32], might be particularly effective against these tumours, as they are in chronic myelogenous leukaemia (another type of tumour with a small number of mutational hits). An alternative conclusion is that LGSCs require many more mutations to develop into full-blown malignancy than our sequencing analysis suggests. Although we can exclude frequent amplifications and deletions through our copy number analysis [12], other alterations, such as translocations and epigenetic changes, are yet to be explored. Accordingly, future genome-wide analyses including miRNA profiles, promoter methylation patterns and mRNA sequencing are warranted to study the contribution of molecular alterations other than sequence mutations and DNA copy number to the development of ovarian LGSC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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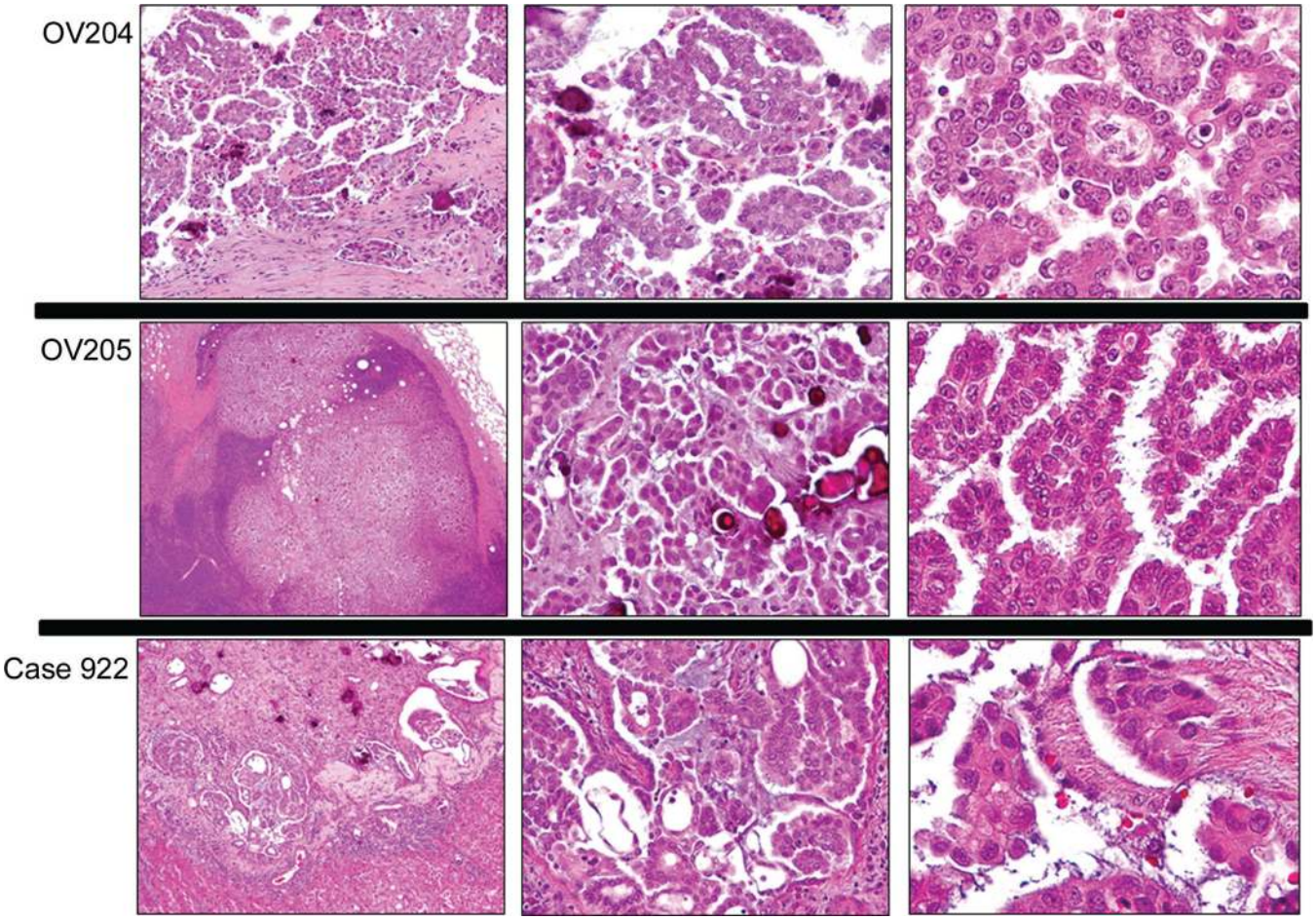

Figure 1.
The morphological features from haematoxylin and eosin-stained sections of three advanced-stage (FIGO stages IIIc and IV), low-grade serous carcinomas of the ovary. All the three cases harboured V600E *BRAF* mutations. (Left panels) Low-magnification views of low-grade serous carcinoma cells metastasizing to the peritoneal wall (OV204 and case 922) or a para-aortic lymph node (OV205). (Middle panels) Medium-magnification views of the tumour cells, exhibiting the characteristic micropapillary architecture with scattered calcification depositions (so-called 'psammoma bodies'). (Right panels) High-magnification views revealing the cytological features of those tumours that are characterized by 'low-grade' or 'grade 1' nuclei, including relatively small and homogeneous nuclear contours and undetectable mitotic figures.

Table 1

All mutations identified in the discovery screen, except in OV207

Sample	Gene	Transcript Accession No.	Nucleotide (genomic)	Nucleotide (cDNA)	Amino acid (protein)	Mutation type	Confirmed by Sanger
OV208PT	*ABCD3*	CCDS749.1	g.chr1 : 94656651C > T	c.29C > T	p.10A > V	Missense	Yes
OV206PT	*ACSM2B*	CCDS32401.1	g.chr16 : 20399695C > T	c.1460 > C > T	p.487A > V	Missense	No
OV209PT	*ADAM22*	CCDS43608.1	g.chr7 : 87595852G > A	IVS8-1G > A	NA	Splice site	Yes
OV204PT	*ADCY5*	CCDS3022.1	g.chr3 : 124554009-12dupATGC	c.1241-44dupGCAT	NA	Frameshift	Yes
OV208PT	*AK3L1*	CCDS629.1	g.chr1 : 65463144G > A	IVS4+3G > A	NA	Splice site	Yes
OV208PT	*AKAP1*	CCDS11594.1	g.chr17 : 52538770G > A	c.946 > G > A	p.316G > S	Missense	No
OV208PT	*ARID1A*	CCDS285.1	g.chr1 : 26973548-51dupCAGC	c.4243-46dupCAGC	NA	Frameshift	Yes
OV202PT	*ASCC3*	CCDS5046.1	g.chr6 : 101207408G > A	c.2902 > C > T	p.968R > C	Missense	No
OV208PT	*ASTE1*	CCDS3068.1	g.chr3 : 132215737 delT	c.2224delA	NA	Frameshift	No
OV208PT	*ATP1B4*	CCDS14598.1	g.chrX:119393449C > T	c.745C > T	p.249R > W	Missense	Yes
OV208PT	*ATP8B2*	CCDS1066.1	g.chr1 : 152570164C > T	c.320C > T	p.107S > F	Missense	Yes
OV206PT	*BAIAP2L2*	CCDS43018.1	g.chr22 : 36823070G > T	c.527 > C > A	p.176A > D	Missense	No
OV204PT	*BBS7*	CCDS3724.1	g.chr4 : 122973994C > T	c.1518G > A	p.506M > I	Missense	Yes
OV204PT	*BRAF*	CCDS5863.1	g.chr7 : 140099605A > T	c.1799T > A	p.600V > E	Missense	Yes
OV208PT	*C13orf23*	CCDS9368.2	g.chr13 : 38506336 delA	IVS1-3delT	NA	Splice site	No
OV208PT	*C14orf106*	CCDS9684.1	g.chr14 : 44763010G > A	c.2530C > T	p.844Q > X	Nonsense	Yes
OV204PT	*C1QTNF1*	CCDS11761.1	g.chr17 : 74555304G > T	c.385G > T	p.129G > W	Missense	Yes
OV204PT	*CCDC142*	CCDS1945.1	g.chr2 : 74555389T > C	c.2024A > G	p.675N > S	Missense	Yes
OV208PT	*CCDC33*	CCDS42058.1	g.chr15 : 72414457C > T	c.2114 > C > T	p.705S > L	Missense	No
OV205PT	*CCDC76*	CCDS765.1	g.chr1 : 100378997C > T	c.503C > T	p.168A > V	Missense	Yes
OV209PT	*CDC27*	CCDS11509.1	g.chr17 : 42571114T > A	c.1694 > A > T	p.565N > I	Missense	No
OV209PT	*CDH2*	CCDS11891.1	g.chr18 : 23827463G > T	c.1157C > A	p.386T > K	Missense	Yes
OV209PT	*COL19A1*	CCDS4970.1	g.chr6 : 70913458C > A	c.1849C > A	p.617P > T	Missense	Yes
OV208PT	*CYLC2*	CCDS35085.1	g.chr9 : 104807455G > C	c.721G > C	p.241E > Q	Missense	Yes
OV208PT	*DDC*	CCDS5511.1	g.chr7 : 50564474G > A	c.496C > T	p.166R > W	Missense	Yes
OV203PT	*DGKH*	CCDS9381.1	g.chr13 : 41678241G > T	c.2560G > T	p.854A > S	Missense	Yes

Sample	Gene	Transcript Accession No.	Nucleotide (genomic)	Nucleotide (cDNA)	Amino acid (protein)	Mutation type	Confirmed by Sanger
OV202PT	*EMR3*	CCDS12315.1	g.chr19 : 14591285T > C	IVS15-2A > G	NA	Splice site	Yes
OV208PT	*EXPH5*	CCDS8341.1	g.chr11 : 107886124 G > C	c.5320C > G	p.1774L > V	Missense	Yes
OV209PT	*FAM65C*	CCDS13431.2	g.chr20 : 48646191G > A	c.1795C > T	p.599P > S	Missense	Yes
OV208PT	*FBN1*	CCDS32232.1	g.chr15 : 46692561C > T	c.185G > A	p.62R > H	Missense	Yes
OV204PT	*FSTL5*	CCDS3802.1	g.chr4 : 162678870G > A	c.1210C > T	p.404R > C	Missense	Yes
OV206PT	*GNRH2*	CCDS13040.1	g.chr20 : 2974356-60dupGCCCC	c.337-41dupGCCCC	NA	Frameshift	No
OV209PT	*HEPACAM*	CCDS8456.1	g.chr11 : 124299968C > T	c.293G > A	p.98R > H	Missense	Yes
OV205PT	*IP6K2*	CCDS2777.1	g.chr3 : 48701018G > A	c.973C > T	p.325R > C	Missense	Yes
OV209PT	*KCNJ12*	CCDS11219.1	g.chr17 : 21260469G > A	c.1222 > G > A	p.408A > T	Missense	No
OV208PT	*KIFC2*	CCDS6427.1	g.chr8 : 145663014C > G	c.163C > G	p.55L > V	Missense	Yes
OV203PT	*KLHL11*	CCDS11411.1	g.chr17 : 37265006G > T	c.639C > A	p.213H > Q	Missense	Yes
OV209PT	*KPRP*	CCDS30862.1	g.chr1 : 151000349G > A	c.1661G > A	p.554R > Q	Missense	Yes
OV209PT	*KRAS*	CCDS8703.1	g.chr12 : 25289551C > T	c.35G > A	p.12G > D	Missense	Yes
OV209PT	*LAMP2*	CCDS14600.1	g.chrX:119457081C > T	c.1189G > A	p.397V > I	Missense	Yes
OV205PT	*LILRA2*	CCDS12900.1	g.chr19 : 59779118C > T	c.985C > T	p.329Q > X	Nonsense	Yes
OV209PT	*LRIG1*	CCDS33783.1	g.chr3 : 66513792G > A	c.2954C > T	p.985A > V	Missense	Yes
OV209PT	*LUC7L*	CCDS32348.1	g.chr16 : 179998C > T	c.944 > G > A	p.315R > Q	Missense	No
OV205PT	*MARCO*	CCDS2124.1	g.chr2 : 119456294C > T	c.994C > T	p.332R > X	Nonsense	Yes
OV203PT	*MCM7*	CCDS5683.1	g.chr7 : 99533212C > T	c.1078G > A	p.360G > R	Missense	Yes
OV208PT	*MYH7*	CCDS9601.1	g.chr14 : 22968919G > T	c.1043C > A	p.348S > Y	Missense	Yes
OV202PT	*NCK1*	CCDS3092.1	g.chr3 : 138149854-6delAAA	c.1003-5delAAA	NA	In-frame deletion	Yes
OV209PT	*NLRP3*	CCDS1632.1	g.chr1 : 245653904G > A	c.536G > A	p.197S > N	Missense	Yes
OV209PT	*OR6K2*	CCDS30902.1	g.chr1 : 156936939A > T	c.128T > A	p.43L > Q	Missense	Yes
OV204PT	*PBX1*	CCDS1246.1	g.chr1 : 163055986C > T	c.1051C > T	p.351Q > X	Nonsense	Yes
OV209PT	*PCDHG*	CCDS4260.1	g.chr5 : 140792593G > A	c.2083G > A	p.695V > I	Missense	Yes
OV209PT	*PHF20*	CCDS13268.1	g.chr20 : 33968975G > T	c.1981G > T	p.661E > X	Nonsense	Yes
OV208PT	*PIK3CA*	CCDS43171.1	g.chr3 : 180404242G > A	c.1030G > A	p.344V > M	Missense	Yes
OV205PT	*PNMA5*	CCDS14718.1	g.chrX:151910416C > A	c.383G > T	p.128S > I	Missense	Yes

Sample	Gene	Transcript Accession No.	Nucleotide (genomic)	Nucleotide (cDNA)	Amino acid (protein)	Mutation type	Confirmed by Sanger
OV205PT	*POLQ*	CCDS33833.1	g.chr3 : 122691680A > T	c.2788T > A	p.930S > T	Missense	Yes
OV208PT	*PRKRA*	CCDS2279.1	g.chr2 : 179005230G > A	IVS7-3 > C > T	NA	Splice site	No
OV209PT	*PRRX1*	CCDS1290.1	g.chr1 : 168971937C > G	c.724C > G	p.242P > A	Missense	Yes
OV208PT	*RBL2*	CCDS10748.1	g.chr16 : 52058534C > T	c.1927C > T	p.643P > S	Missense	Yes
OV208PT	*REPS2*	CCDS14180.2	g.chrX:16980489G > A	c.971G > A	p.324 W > X	Nonsense	Yes
OV209PT	*RNF214*	CCDS41720.1	g.chr11 : 116614795G > T	c.376G > T	p.126E > X	Nonsense	Yes
OV208PT	*S100PBP*	CCDS30666.1	g.chr1 : 33064901C > G	c.614C > G	p.205S > C	Missense	Yes
OV208PT	*SDSL*	CCDS9170.1	g.chr12 : 112350202A > G	c.32A > G	p.11Q > R	Missense	Yes
OV205PT	*SLC9A6*	CCDS44003.1	g.chrX:134908689T > A	c.689 > T > A	p.230M > K	Missense	No
OV209PT	*SPATA5*	CCDS3730.1	g.chr4 : 124068326G > T	c.251G > T	p.84R > L	Missense	Yes
OV208PT	*SPOP*	CCDS11551.1	g.chr17 : 45054368C > T	c.139G > A	p.47E > K	Missense	Yes
OV208PT	*SRCAP*	CCDS10689.2	g.chr16 : 30638999C > T	c.2833C > T	p.945R > X	Nonsense	Yes
OV203PT	*SRP72*	CCDS3506.1	g.chr4 : 57052353G > A	IVS15-1G > A	NA	Splice site	Yes
OV203PT	*STYK1*	CCDS8629.1	g.chr12 : 10668616-18delAAA	c.827-29delTTT	NA	In-frame deletion	Yes
OV205PT	*TCF25*	CCDS10987.1	g.chr16 : 88478529 dupA	c.393dupA	NA	Frameshift	No
OV204PT	*TMEM202*	CCDS32287.1	g.chr15 : 70486577T > C	c.584T > C	p.195L > P	Missense	Yes
OV208PT	*TNNI3K-FPGT*	CCDS663.1	g.chr1 : 74442707A > T	c.388A > T	p.130I > F	Missense	Yes
OV204PT	*TPO*	CCDS1643.1	g.chr2 : 1523465C > A	c.2711C > A	p.904T > N	Missense	Yes
OV203PT	*TRIM31*	CCDS34374.1	g.chr6 : 30179332G > A	c.1238C > T	p.413T > I	Missense	Yes
OV209PT	*TSG101*	CCDS7842.1	g.chr11 : 18480703delA	IVS6-3delT	NA	Splice site	Yes
OV204PT	*TSPAN11*	CCDS31765.1	g.chr12 : 31008218C > T	c.275C > T	p.92T > M	Missense	Yes
OV209PT	*TSPAN11*	CCDS31765.1	g.chr12 : 31027266G > A	c.616G > A	p.206G > R	Missense	Yes
OV204PT	*VDR*	CCDS8757.1	g.chr12 : 46545141G > C	c.233 > C > G	p.78A > G	Missense	No
OV208PT	*WWTR1*	CCDS3144.1	g.chr3 : 150857512G > T	c.272C > A	p.91P > H	Missense	Yes
OV203PT	*XIRP2*	CCDS42769.1	g.chr2 : 167814105G > C	c.7957G > C	p.2653D > H	Missense	Yes
OV208PT	*ZFYVE16*	CCDS4050.1	g.chr5 : 79769548G > C	c.1288G > C	p.430E > Q	Missense	Yes
OV208PT	*ZFYVE16*	CCDS4050.1	g.chr:579769674G > A	c.1414G > A	p.472D > N	Missense	Yes
OV209PT	*ZNF572*	CCDS6354.1	g.chr8 : 126057095A > T	c.32A > T	p.11D > V	Missense	Yes

* All coordinates refer to the human reference genome hg18 release (NCBI 36.1, March 2006). NA, not applicable.

Table 2

Summary of mutational analysis of the 15 genes selected for validation

set	case	diagnosis	age	stage	follow up	*KRAS*	*BRAF*	*PIK3CA*	*ARID1A*	*TSPAN11*	*STYK1*	*CCDC76*	*RNF214*	*SMARCA4*	*SPATA5*	*RBL2*	*PPP2R1A*	*PIK3C2A*	*DDC*	*C14orf106*
discovery	OV202	LGS	32	IIIC	LWD 74 m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
discovery	OV203	LGS	44	IV	N/A	wt	wt	wt	wt	wt	827delTTT	wt	wt	wt	wt	wt	wt	wt	wt	wt
discovery	OV204	LGS	46	IIIC	LWD 60 m	wt	600V/E	wt	wt	92T/M	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
discovery	OV205	LGS	72	IIIC	DOD 42 m	wt	600V/E	wt	wt	wt	wt	168A/V	wt	wt	wt	wt	wt	wt	wt	wt
discovery	OV206	LGS	35	IIIC	LWD 26 m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
discovery	OV207	LG (S+EM)	49	IC	N/A	wt	wt	88R/Q	5543insG; 6415delC	wt	wt	wt	239R/H	539R/C	wt	wt	183R/Q	572R/X	39R/W	wt
discovery	OV208	LG (S+CC)	49	IV	DOD 5 m	wt	wt	344V/M	4247ins CAGC	wt	wt	wt	wt	wt	wt	643P/S	wt	wt	166R/W	844Q/X
discovery	OV209	LGS	59	IIIC	LWD 54 m	12G/D	wt	wt	wt	206G/R	wt	wt	126E/X	wt	84R/L	wt	wt	wt	wt	wt
validation	543	LGS	25	IIIB	LWD 60 m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	10089	LGS	45	IIIC	LWD 3 m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	922	LGS	33	II	LWD 23 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	116	LGS	62	IIIC	DOD 35 m	wt	wt	345N/K	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	1107	LGS	45	IIIC	N/A	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	406	LGS	33	IIIC	LWD 12 m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	610	LGS	48	IIIC	LWD 16 m	12G/V	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	701	LGS	56	IIIC	LWD 10 m	12G/D	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	609	LGS	46	IIIC	DOD 14 m	12G/D	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	976	SBT*	51	N/A	ND 24 m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	832	SBT*	54	N/A	ND 22 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	725	SBT	35	N/A	N/A	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	925	SBT	25	N/A	ND 26 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	1017	SBT	37	N/A	ND 32 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	869	SBT	47	N/A	ND 20 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	345	SBT	41	N/A	N/A	12G/D	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	623	SBT	67	N/A	ND 8m	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
validation	485	SBT	39	N/A	LWD 90 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt

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set	case	diagnosis	age	stage	follow up	*KRAS*	*BRAF*	*PIK3CA*	*ARID1A*	*TSPAN11*	*STYK1*	*CCDC76*	*RNF214*	*SMARCA4*	*SPATA5*	*RBL2*	*PPP2R1A*	*PIK3C2A*	*DDC*	*C14orf106*
validation	1007	SBT	27	N/A	ND 4 m	wt	600V/E	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt

LGS: low-grade serous carcinoma; SBT: serous borderline tumor; EM: endometrioid; CC: clear cell; S: serous; N/A: not available; DOD: dead of disease; LWD: live with disease; ND: no evidence of disease; wt: wild-type.

* containing non-invasive low-grade (micropapillary) serous carcinoma.