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ORIGINAL RESEARCH

A novel mutation panel for predicting etoposide resistance in small-cell lung cancer

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Purpose: Platinum-based chemotherapy, consisting of etoposide and cisplatin (EP), has been the cornerstone of therapy for extensive-stage small-cell lung cancer (ES-SCLC) for decades. Despite the marked initial sensitivity of SCLC to chemotherapy, EP regimens cannot avoid the emergence of drug resistance in clinical practice. With the rise of new chemotherapy regimens in recent years and the primary resistance or insensitivity of ES-SCLC to EP regimens, it is desirable to be able to identify patients with resistant or insensitive ES-SCLC.

Methods: The sequencing and drug sensitivity data of SCLC cell lines were provided by The Genomics of Drug Sensitivity in Cancer Project (GDSC). The data regarding sensitivity to etoposide of 54 SCLC cell lines were analyzed, and etoposide-sensitive cell lines and etoposide-resistant cell lines were differentiated according to the IC50 values defined by the GDSC. ROC curve analysis was performed on all mutations and combinations of mutations to select the optimal panel to predict resistance to etoposide.

Results: ROC analysis of etoposide resistance revealed that the most significant single gene mutation indicating resistance to etoposide was *CSMD3*, and the accuracy of predicting resistance to etoposide proved to be the highest when there was any mutation in *CSMD3/PCLO/RYR1/EPB41L3*, area under the curve =0.804 (95% confidence interval: 0.679–0.930, $P<0.001$).

Conclusion: This study found that a panel with four genes (*CSMD3, EPB41L3, PCLO, and RYR1*) can accurately predict sensitivity to etoposide. These findings provide new insights into the overall treatment for patients with ES-SCLC that is resistant or insensitive to etoposide.

Keywords: small-cell lung carcinoma, etoposide, EP regimens, IP regimens, gene mutation

Introduction

In recent years, humans have made significant progress in the early detection, early diagnosis, early treatment, and even prevention of cancer. However, lung cancer is the most commonly diagnosed cancer (11.6%) and the leading cause of cancer-related death (18.4%) worldwide.[1] Currently, there are approximately 2.1 million lung cancer patients worldwide.[1] Approximately 12–15% of new lung cancer patients are diagnosed with small-cell lung cancer (SCLC).[2,3] According to the latest National Comprehensive Cancer Network (NCCN) Guidelines, an estimated 29,654 new cases of SCLC occurred in the United States in 2017.[4,5] Studies have shown that the incidence of SCLC is attributable to cigarette smoking, and the smoking pack-years increases, so does the risk of SCLC. Ninety percent of patients with SCLC have been or are currently smokers, and smoking duration is positively associated with an increased risk of SCLC.[6,7] In addition, SCLC is characterized by a high growth fraction, a high degree of malignancy, and the early development of

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widespread metastases.[8,9] The 5-year survival rate in patients with SCLC is only 6.6%. Currently, SCLC is divided into limited-stage SCLC (LS-SCLC) and extensive-stage SCLC (ES-SCLC). Unfortunately, the 5-year survival rates are only 1.6% and 12.1% for patients with ES-SCLC (1/3) and ES-SCLC (2/3),[8–11] respectively.

At present, surgery is one of the main methods of cancer treatment, but it is rarely used in the treatment of patients with SCLC. It is only suitable for a small number of stage I patients with SCLC (2%-5%) who do not have mediastinal lymph node metastasis. In the past few decades, a platinum compound in combination with the topoisomerase-II inhibitor etoposide beyond 4 to 6 cycles of chemotherapy (EP) has become the cornerstone of treatment for patients with ES-SCLC for palliative care.[11–13] In recent years, the chemotherapy for ES-SCLC has mainly been irinotecan, cisplatin (IP) and EP regimens.[14] Despite the substantial initial sensitivity of SCLC to chemotherapy in the early stages of treatment, more than 90% of patients eventually develop clinical drug resistance and die as a result of relapse.[8,9] At present, there is a great deal of controversy about the therapeutic effect and safety tolerance of IP and EP in the treatment of ES-SCLC. In 2002, a randomized, multicenter, phase III trial (J9511) performed in Japan reported that patients with ES-SCLC who were treated with IP experienced a median survival of 12.8 months compared with 9.4 months for patients treated with EP (P=0.002). In addition, the 1-year survival rates were 58.4% vs 37.7% and the median progression-free survival (PFS) rates were 12.8 months vs 9.4 months in the IP and EP groups, respectively.[15] Furthermore, Hermes et al studied 220 patients with ES-SCLC, and the results showed that the median overall survival (OS) was slightly higher in those receiving IP than in those receiving EP (8.5 months vs 7.1 months, P=0.04).[16] However, it is surprising that there were no significant differences in the efficacy and survival of the IP and EP groups in 4 subsequent phase III trials.[17–20] In a cohort study from Korea, the median OS and median PFS of patients with ES-SCLC treated with IP were 10.9 months and 6.5 months, respectively, whereas the median OS and PFS in the EP arm were 10.3 months (P=0.120) and 5.8 months (P=0.115), respectively. Similarly, no significant differences were observed in the 1- and 2-year survival rates in the IP versus EP groups. In the subgroup analysis, males, patients <65 years old and patients with Eastern Cooperative Oncology Group performance status (ECOG PS) ≤1 were treated with IP or EP, and the two groups had significant therapeutic differences. In addition, there was a significant difference in the objective response rate (ORR) between the IP group and the EP group (62.4% vs 48.2%, P=0.006).[21]

Currently, 4 to 6 cycles EP is the standard therapy widely used for a majority of SCLC in the clinic, with an ORR of 50%-80%.[22] However, the median OS of patients with ES-SCLC is only 9 months, with only 2% of patients surviving after 5 years.[14,23] Although SCLC usually responds well to chemotherapy regimens in the early stages of treatment, subsequent clinical drug resistance and disease recurrence occur in more than 90% of patients.[8,9] This may be due to the existence of cancer stem cells that are relatively resistant to cytotoxic therapy. Chemotherapy cannot destroy residual tumor cells, leading to a high recurrence rate and a high drug resistance rate in SCLC.[24] Primary resistance or acquired resistance to chemotherapy is a major factor in the poor prognosis of patients with lung cancer.[25–27] In the drug sensitivity data from GDSC, we found that the IC50 of etoposide in the 54 SCLC cell lines ranged from 0.242 μM to 319 μM, and the drug resistance cut-off value provided by the website was 16 μM. In total, 65% of patients have SCLC that is sensitive to etoposide, which is close to the response rate for etoposide.[28] Therefore, if we are able to select patients with ES-SLCL that is not sensitive to etoposide before treating them with standard chemotherapy, we could choose a different chemotherapy regimen to treat these patients, hopefully improving survival outcomes in those ES-SCLC patients. Survival time was significantly improved with the new chemotherapy compared with EP. However, there is currently no clinically relevant prediction factor and screening for appropriate means of insensitivity to etoposide.

To date, a growing number of studies have shown that the emergence of primary or acquired platinum and Topoisomerase Inhibitors resistance in EP is associated with certain gene expression changes or/and gene mutations.[29] Chiu et al[30] found that *FBXL7* is a biomarker of poor prognosis in patients with ovarian cancer. A high expression level of *FBXL7* is positively associated with a low survival rate in ovarian cancer patients, and the FBXL7 mRNA level and ovarian cancer cell line paclitaxel (PTX) IC50 values were positively correlated, leading to the speculation that the upregulation of *FBXL7* expression results in resistant ovarian cancer cell lines. In addition, Chiu et al[31] detected the transcriptional level of the shared gene in HCC38 (PTX-sensitive) and MDA-MB436 (PTX-resistant) TNBC cells posttreatment with paclitaxel. They found that the downregulation of miR-1180 may regulate *OTUD7B*, ultimately negatively regulating the NF-κB-Lin28 axis. This in turn triggers Let-7 microRNA-mediated caspase-3 downregulation, ultimately leading to resistance to PTX. Based on these findings, the sensitivity and drug resistance of tumor cells to chemotherapy can be predicted by gene expression levels. Thus, patients with ES-SLCL that is sensitive or insensitive to chemotherapy can be further distinguished. We hope that the sensitivity of ES-SCLC to etoposide can be predicted by gene mutation panels, allowing the selection of patients with ES-SCLC that is insensitive to etoposide before standard chemotherapy is administered and the development of personalized, precise chemotherapy to extend patients' OS and improve their quality of life (QOL).

To this end, we analyzed the sequencing and drug sensitivity data for a SCLC cell line through the GDSC database to determine whether mutations can predict the primary resistance to etoposide and try to explain the potential underlying mechanism to provide first-line treatment recommendations for patients with ES-SCLC.

Methods

Drug response, gene expression and mutation data

The natural logarithm half maximal inhibitory concentration (IC50) of all selected erlotinib-related cell lines were obtained from the GDSC (https://www.cancerrxgene.org/). Robust Multichip Average (RMA) normalized expression data from the Affymetrix Human Genome U219 array and gene mutation information found in cell lines by Illumina HiSeq 2000 whole-exome sequencing (WES) were downloaded from the GDSC.

Screening of mutated resistance genes

There were 54 SCLC cell lines in the GDSC with drug sensitivity data for etoposide. The GDSC site defined etoposide-resistant cell lines as those with IC50 values ≥16 μM and etoposide-sensitive cell lines as those with IC50 values <16 μM. ROC curve analysis was performed for all mutations, and the cell lines with areas under the curve (AUCs) >0.5 were selected and randomly combined; then, resistance to etoposide was predicted by the combined mutation panels. The Youden Index values obtained by various combined ROC analyses were sorted to select the best combination.

Statistical analysis

The IC50 distribution for etoposide in various cell lines was obtained with the GDSC web tool. ROC analysis and mapping were performed with SPSS 21.0 (IBM SPSS Statistics, IBM Corporation); mutation and gene expression data were analyzed and mapped with the maftools[32] and limma packages[33] in R. In the differential analysis of the gene expression profiles, $P<0.05$ and $FC>1.5$ or$FC<2/3$ were considered to indicate significant differences. The survival analysis was with the log-rank test after the Kaplan-Meier analysis to investigate the predictive ability of a mutation panel with regard to survival. Gene Ontology (GO) annotation analysis and KEGG pathway enrichment analysis of the differentially expressed genes (DEGs) in this study were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/).

Results

The sensitivity of cancer cell lines to drugs is mainly expressed as the IC50 value, which refers to the concentration of drug that kills half of the tumor cells in vitro. Because the drug concentration is diluted to 1/10 or 1/100, we used lnIC50 values to distinguish between resistant or sensitive cell lines. Based on the GDCS 7.0 database (updated on March 20, 2018), there are 64 SCLC cell lines, but only 54 of them have etoposide susceptibility data (drug sensitivity data), WES mutation data and RNA Seq data.

Using the GDSC website tools, we obtained the IC50 distribution for etoposide by tissue type (Figure 1A). We found that most of the tumors are sensitive to etoposide, and the IC50 values of most cell SCLC lines indicate that they are sensitive to etoposide. By analyzing the IC50 values of the 54 SCLC cell lines shown in Figure 1B, we found that there are 35 cell lines that are sensitive to etoposide, accounting for 64.8% of the total, and their median and mean IC50 values were 2.06 μM (range: 0.242–15.2 μM) and 4.02±4.07 μM, respectively. In total, 19 strains were resistant to etoposide, accounting for 35.2% of the total, and their median and mean IC50 values were 50.0 μM (range: 16.4–319.0 μM) and 71.9 ±71.8 μM, respectively. The raw data for the IC50 values of all cell lines with regard to etoposide can be found in Table S1.

After sorting the IC50 values for etoposide, we found that in the mutation landscape of the 54 SCLC cell lines (Figure 2), the genes with the highest mutation frequencies were *TP53* (91%), *TTN* (78%) and *Rb1* (70%). Among them, *TP53* and *TTN* mutations were mainly missense mutations, while the *Rb1* mutations were mainly nonsense and splice mutations.

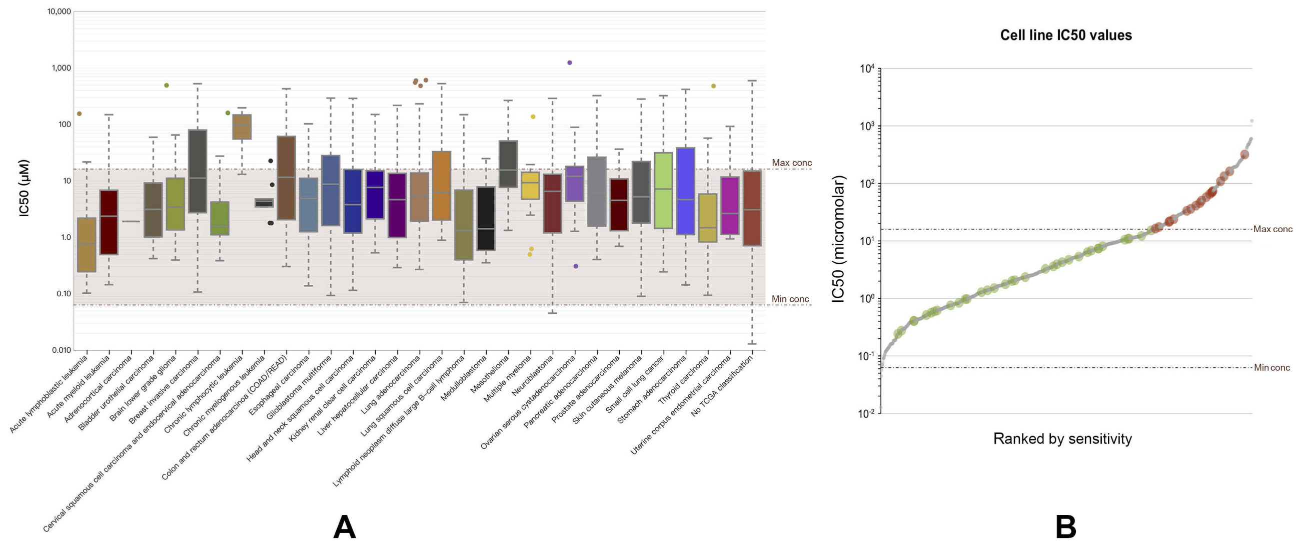

Figure 1 (**A**) IC50 distribution for etoposide by tissue type. (**B**) The scatter plot of IC50 distribution for etoposide of 54 SCLC cell lines.
Abbreviation: IC50, half maximal inhibitory concentration.

We performed an ROC analysis of to predict etoposide resistance using all mutated genes (see Table S2). From the ROC curves, we found that the most significant single gene mutation associated with resistance to etoposide was *CSMD3*, with an AUC of 0.697 (P=0.016) (Table 1). By experimenting with different combinations, we found that when any mutations occurred in *CSMD3/PCLO/RYR1/EPB41L3*, the accuracy of predicting resistance to etoposide was the highest (AUC=0.804, 95% CI: 0.679–0.930, $P<0.001$) (Table 1). The ROC curve results of the panel composed of *CSMD3/PCLO/RYR1/EPB41L3* and the individual genes are shown in Figure 3A.

We performed a log-rank test with the Kaplan–Meier plots according to mutations and clinical follow-up data in 110 SCLCs published by George et al[34] In addition, we found a significantly lower average survival time in patients with CLC with any mutation in CSMD3/PCLO/RYR1/EPB41L3 than in those with no mutations in all four genes (35.6±5.3 months vs 76.7±12.1 months, P=0.040) (Figure 3B). By analyzing significantly enriched KEGG pathways of DEGs, we found that there was a significant association between both CSMD3 and RYR1 mutations and MAPK signaling pathway (P=0.015 and P=0.023, respectively) (Table 2).

Discussion

EP has been the most common therapy for ES-SCLC for decades. As a standard treatment, it can inhibit tumor proliferation, relieve clinical symptoms, and achieve ideal results.[13,34–37] We found that 19 (35.2%) of the 54 SCLC cell lines were insensitive to etoposide according to the data from the GDSC. Currently, the clinically accepted ORR of EP is 50–80%.[23] Based on the above findings, the majority of patients with SCLC do not receive survival benefits from EP, indicating that screening for patients with primary resistance to etoposide is necessary. Therefore, this study further analyzed the mutation, gene expression and etoposide sensitivity data of 54 ES-SCLC cell lines obtained from the GDSC. We identified four genes, namely, *CSMD3, EPB41L3, PCLO*, and *RYR1*; mutations in these genes predict resistance to etoposide. The predictive sensitivity this four-gene panel for resistance to etoposide is as high as 85%, with 77.8% accuracy when screening for patients with primary etoposide resistance. In addition, the ROC showed an AUC of 0.804 (95% CI 0.679–0.930), and the model was considered to have a high degree of confidence.

Recently, a small phase III trial performed in Japan compared the efficacy of IP and EP in patients with ES-SCLC[15]. The trial results showed a higher median OS (12.8 months vs 9.4 months), 1-year survival rate (58.4% vs 37.7%) and 2-year survival rate (19.5% vs 5.2%) after IP than after EP. In addition, Hermes et al[16] studied 220 patients with ES-SCLC, and the results showed a longer median OS resulting from the IP regimen compared with the EP regimen (8.5 months vs 7.1 months, P=0.04).

We analyzed the data and found that mutations in both *CSMD3* and *RYR1* can cause the activation of the downstream MAPK signaling pathway (Figure 4). In addition, Liu et al[36] found that etoposide activates the MAPK/ERK signaling pathway, inhibits p53 expression and enhances c-Myc expression

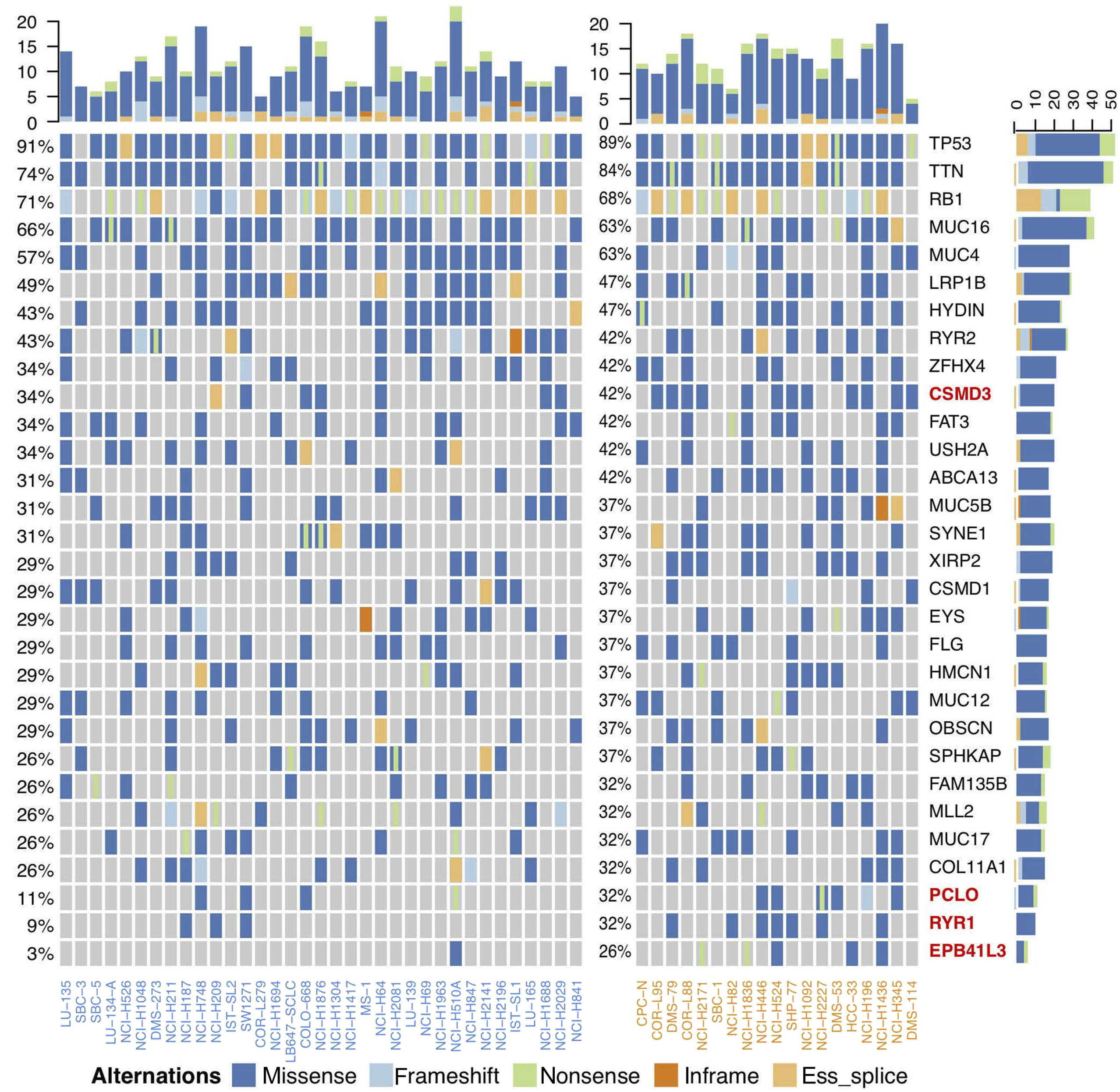

Figure 2 Mutation landscape of 54 SCLC cell lines.
Abbreviation: SCLC, small-cell lung cancer.

Table 1 Receiver operator characteristic curve analysis for four-gene panel and four genes separately to etoposide resistance status in small-cell lung cancer cell lines

Gene	Area under curve	95% confidence interval	Sensitivity	Specificity	Youden index	*P*-value
CSMD3	0.697	0.546–0.848	0.600	0.794	0.394	0.016
PCLO	0.591	0.429–0.754	0.300	0.882	0.182	0.267
RYR1	0.631	0.469–0.792	0.350	0.912	0.262	0.111
EPB41L3	0.610	0.447–0.774	0.250	0.971	0.221	0.179
Panel	0.804	0.679–0.930	0.850	0.706	0.556	<0.001

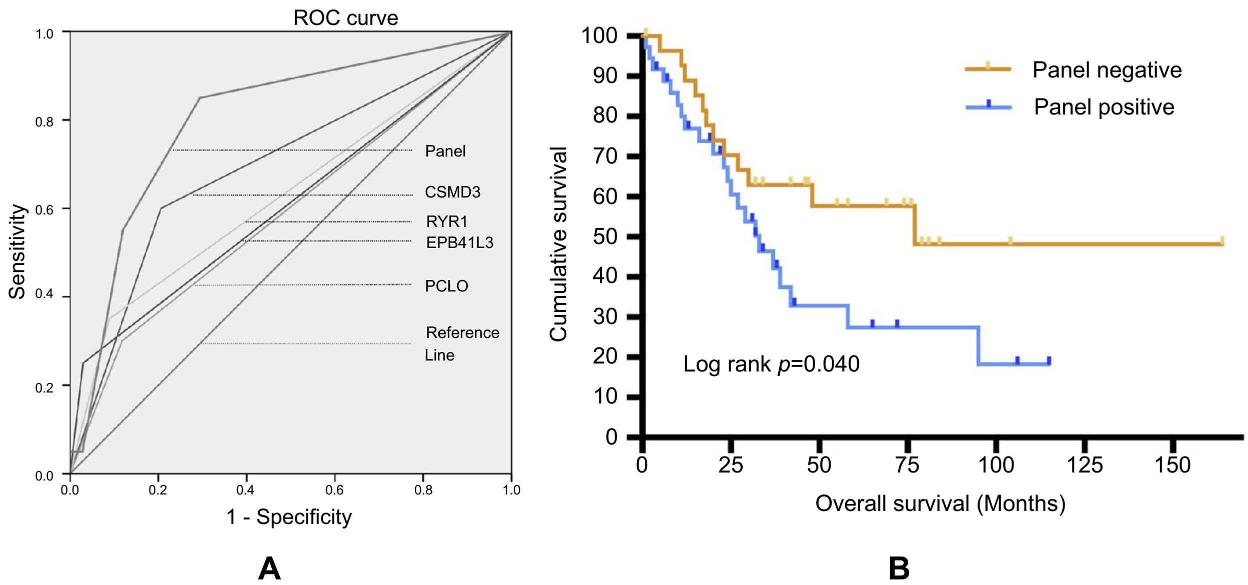

Figure 3 (**A**) ROC curve of the panel and four mutations; (**B**) Kaplan–Meier overall survival analyses for the four-gene panel in clincal trial of SCLC.
Abbreviation: SCLC, small-cell lung cancer.

Table 2 Significantly enriched KEGG pathways of DEGs

Mutation	Term	Count	*P*-value
CSMD3	hsa04142: Lysosome	8	0.002
	hsa04010: MAPK signaling pathway	10	0.015
	hsa05230: Central carbon metabolism in cancer	5	0.016
	hsa04610: Complement and coagulation cascades	5	0.021
	hsa01130: Biosynthesis of antibiotics	8	0.044
EPB41L3	hsa01200: Carbon metabolism	8	0.003
	hsa01130: Biosynthesis of antibiotics	11	0.004
	hsa01100: Metabolic pathways	33	0.010
	hsa00020: Citrate cycle (TCA cycle)	4	0.015
	hsa04730: Long-term depression	5	0.020
	hsa04130: SNARE interactions in vesicular transport	4	0.021
	hsa04720: Long-term potentiation	5	0.028
	hsa03022: Basal transcription factors	4	0.044
	hsa04726: Serotonergic synapse	6	0.045
PCLO	hsa04810: Regulation of actin cytoskeleton	11	<0.001
	hsa04151: PI3K-Akt signaling pathway	12	0.005
	hsa04510: Focal adhesion	9	0.005
	hsa04512: ECM-receptor interaction	6	0.005
	hsa03320: PPAR signaling pathway	5	0.011
	hsa05205: Proteoglycans in cancer	8	0.016
	hsa05160: Hepatitis C	6	0.031
	hsa05231: Choline metabolism in cancer	5	0.044
RYR1	hsa00500: Starch and sucrose metabolism	3	0.019
	hsa04010: MAPK signaling pathway	6	0.023
	hsa04960: Aldosterone-regulated sodium reabsorption	3	0.026
	hsa00280: Valine, leucine and isoleucine degradation	3	0.037
	hsa01130: Biosynthesis of antibiotics	5	0.048

Abbreviations: MAPK, mitogen activated kinase-like protein; TCA, tricarboxylic acid; SNARE, small NF90 (ILF3) associated RNA E; PI3K-Akt:phosphoinositide-3-kinase/serine threonine kinase; ECM, extracellular matrix; PPAR, peroxisome proliferators-activated receptors.

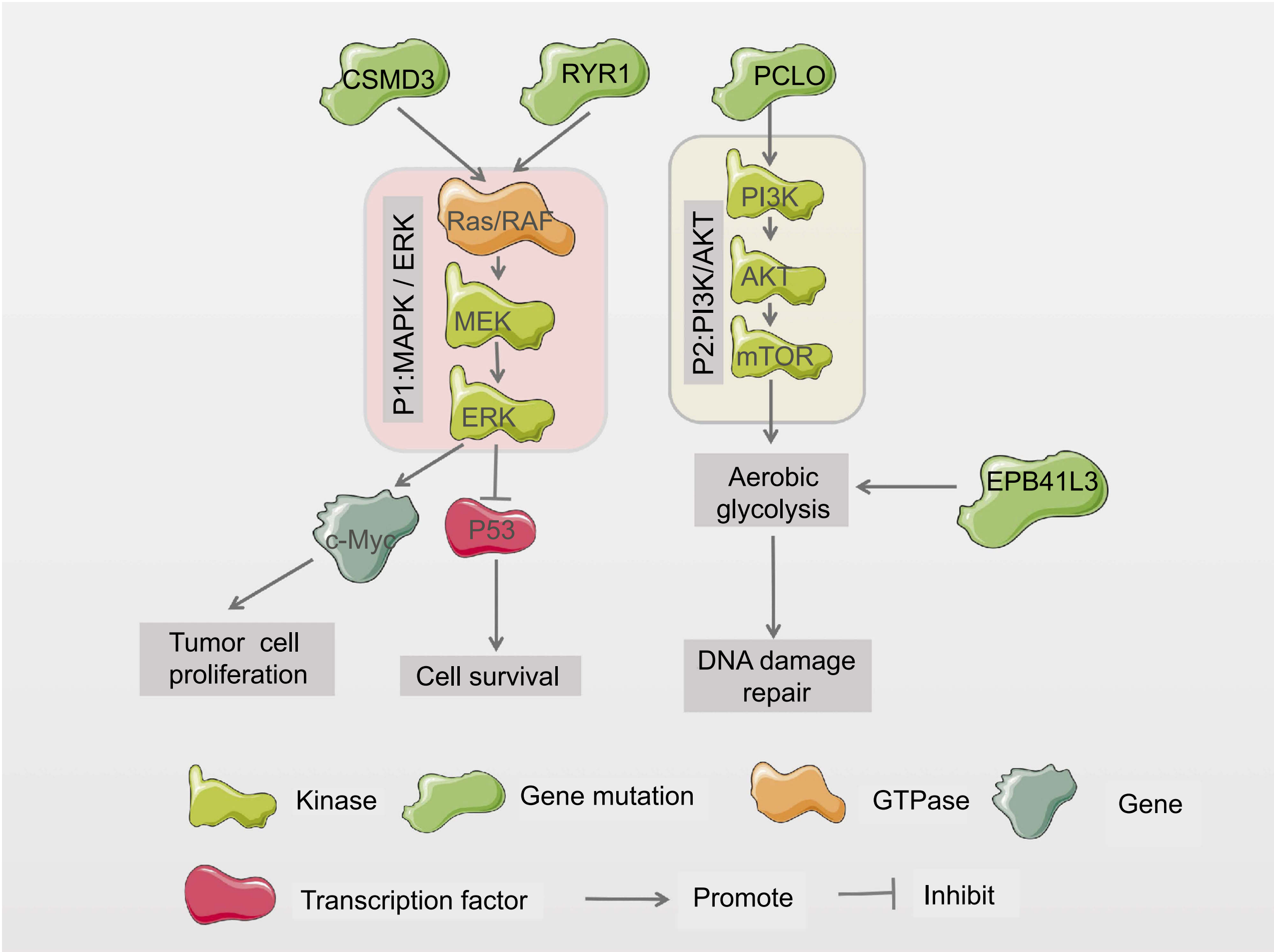

Figure 4 Potential mechanism of the four-gene panel to predict the resistance of etoposide in SCLC.
Abbreviation: SCLC, small-cell lung cancer.

to decrease the sensitivity of gastric cancer cells to chemotherapy in. Therefore, we hypothesized that mutations in the *CSMD3* and *RYR1* genes may cause a significant resistance to etoposide in ES-SCLC via the downstream MAPK signaling pathway. It is well known that etoposide induces DNA double-strand breakage (DSB) and triggers the DNA damage response by activating the ataxia telangiectasia-mutated gene (ATM) DNA repair is a process of energy dissipation, and ATP-dependent chromatin remodeling complexes participate in DSB repair.[37] In aerobic conditions, tumor cells preferentially perform glycolysis rather than providing energy for cell growth through the more efficient oxidative phosphorylation pathway and are therefore characterized by high glucose uptake, glycolysis activity levels and lactic acid content in the metabolites. Glycolysis consumes more glucose but produces less ATP.[38] The PI3K/AKT signaling pathway promotes aerobic glycolysis by upregulating cell surface glucose transporters[39] and glycolytic enzymes in tumor cells.[40,41] Surprisingly, we found that the mutation of the *EPB41L3* gene caused increased activity of the glucose metabolism pathway in tumor cells. Therefore, we speculate that mutations in *EPB41L3* may reduce sensitivity to etoposide through DNA repair in tumor cells. In addition, AKT is involved in the repair of DNA damage caused by genotoxicity, mainly by the action of DNA-dependent protein kinase (DNA-PK), the kinase ATM/ATM and nonhomologous end joining (NHEJ) to repair DSB.[42] Makinoshima et al[43] found that PI3K/AKT/mTOR signaling inhibitors can effectively inhibit the expression of GLUT1 on the cell membrane. They used RNAi to interfere with the expression of GLUT1, ultimately reducing the aerobic glycolysis process and cell proliferation rate. Furthermore, our results suggest that *PCLO* mutations cause activation of the PI3K-Akt pathway, so we hypothesized that *PCLO* mutations may enhance glucose metabolism by activating the PI3K/Akt pathway, thereby enhance the ability of the tumor cell to repair DNA.

Table 3 Completed/ongoing clinical trials of alternative treatment of etoposide in SCLC patients

Drug name	Clincal phase	Comments	NCT No.	Treatment	Pathway/target
Irinotecan	3		NCT00168896	Carboplatin+Irinotecan	Topoisomerase 1
	2		NCT01441349		
	2		NCT01441349	Carboplatin+Sunitinib+Irinotecan	
	2		NCT00695292		
	1		NCT00045604	Cisplatin+Irinotecan+Imatinib	
	1	c-kit positive	NCT00052494		
	2		NCT00248482		
	1		NCT00059761	Cisplatin+Irinotecan	
	2		NCT01441349		
	2		NCT01441349	Cisplatin+Simvastatin+Irinotecan	
	2		NCT00452634		
	2		NCT00546130	Cisplatin+Krestin+Irinotecan	
	2		NCT00118235	Cisplatin+Irinotecan+Bevacizumab	
Bevacizumab	2		NCT00118235	Cisplatin+Irinotecan+Bevacizumab	VEGF
Pemetrexed	2		NCT00051506	Carboplatin+Pemetrexed	TS, DHFR,GARFT
	2		NCT00494026		
	2		NCT00051506	Cisplatin+Pemetrexed	
	2		NCT00475657		
Dimethylxanthenone Acetic Acid (DMXAA)	2		NCT01057342	Carboplatin+Dimethylxanthenone Acetic Acid (DMXAA)+Paclitaxel	DT-diaphorase
Paclitaxel	2		NCT01057342	Carboplatin+Dimethylxanthenone Acetic Acid (DMXAA)+Paclitaxel	Mitosis;Microtubule stabiliser
	2		NCT00454324	Carboplatin+Paclitaxel	
	1		NCT02069158	Carboplatin+Paclitaxel+PF-05212384	
PF-05212384	1		NCT02069158	Carboplatin+Paclitaxel+PF-05212384	PI3K/mTOR;PI3Kα, PI3Kγ,mTOR
Gemcitabine	2		NCT02722369	Carboplatin+Gemcitabine	DNA replication;Pyrimidine antimetabolite
Pegfilgrastim	2	Be able to receive growth factors (G-CSF)	NCT01076504	Carboplatin+Pegfilgrastim+Amrubicin	Granulocyte colony-stimulating factor receptor; Neutrophil elastase
Amrubicin	2	Be able to receive growth factors (G-CSF)	NCT01076504	Carboplatin+Pegfilgrastim+Amrubicin	Topoisomerase 2
Sunitinib	2		NCT00695292	Carboplatin+Sunitinib+Irinotecan	RTK signaling;PDGFR, KIT, VEGFR, FLT3, RET, CSF1R

(*Continued*)

Table 3 (Continued)

Drug name	Clincal phase	Comments	NCT No.	Treatment	Pathway/target
Topotecan	2		NCT00316186	Carboplatin+Topotecan	DNA topoisomerases
	3		NCT00043927	Cisplatin+Topotecan	
	2		NCT00028925	Carboplatin+Topotecan+G-CSF	
Belotecan	3		NCT00826644	Cisplatin+Belotecan	HDAC
Imatinib	2		NCT00248482	Cisplatin+Irinotecan+Imatinib	RTK signaling;ABL, KIT, PDGFR
	1		NCT00045604		
	1	c-kit positive	NCT00052494		
Simvastatin	2		NCT01441349	Cisplatin+Simvastatin+Irinotecan	HMG-CoA Reductase
	2		NCT00452634		
	2		NCT01441349	Carboplatin+Irinotecan+Simvastatin	
Krestin	2		NCT00546130	Cisplatin+Krestin+Irinotecan	Apoptosis;p21(WAF/Cip1)
Sagopilone	2		NCT00359359	Cisplatin+Sagopilone	Microtubule stabiliser

Notes: TS, Thymidylate Synthetase; DHFR, Dihydrofolate Reductase; GARFT, Formylglycinamide Ribotide Amidotransferase; PI3K/mTOR, Phosphoinosmde-3-Kinase/The Mammalian Target of Rapamycin; HMG-CoA, Hydroxy Methylglutaryl Coenzyme A Reductase; RTK, Receptor Tyrosine Kinase; PDGFR, Platelet-Derived Growth Factor Receptors; KIT, KIT proto-oncogene, Receptor Tyrosine Kinase; VEGFR, Vascular Endothelial Growth Factor Receptor; FLT3, Fms Related Tyrosine Kinase; RET, Ret Proto-Oncogene; CSF1R, Colony Stimulating Factor 1 Receptor; HDAC, Histone Deacetylase; ABL, Abl Tyrosine Kinase; p21(WAF/Cip1), Cyclin Dependent Kinase Inhibitor; G-CSF, granulocyte colony stimulating factor; SCLC,small-cell lung cancer.

Identifying outpatients with ES-SCLC that is not sensitive to etoposide and treating them with another combination therapy are important steps in improving the survival of patients with SCLC. Screening for the sensitivity to etoposide in patients with SCLC who are receiving chemotherapy for the first time allows clinicians to use a different combination chemotherapy regimen (Table 3) in these patients to avoid treatment failure due to primary resistance to etoposide. Currently, alternative treatment options that are commonly used in clinical practice include IP protocols, platinum-based drugs plus paclitaxel, and IP plus sunitinib. A phase II clinical trial (NCT00454324) on the use of a platinum-based compound plus paclitaxel in patients with ES-SCLC has shown good efficacy.[44] In a phase II clinical trial (NCT00695292),[45] sunitinib combined with IP for patients with ES-SCLC showed potential clinical efficacy and safety, with an ORR of 59%, a one-year survival rate of 54% and a median PFS of 7.6 months. In recent years, combinations of various chemotherapy regimens have been shown to provide excellent survival advantages in patients with ES-SCLC. It may be possible to classify patients by adding inclusion criteria and then use a more specific new chemotherapy regimen as a clinical treatment to achieve individualized and precise treatment of ES-SCLC patients, overcoming the treatment bottleneck for patients with ES-SCLC that is resistant to EP and ultimately prolonging their survival time and improving their QOL.

There were some limitations in this study. First, the most suitable alternative drug at present is irinotecan. GDSC does not provide data regarding the sensitivity to irinotecan, and the sensitivity of etoposide-resistant ES-SCLC to irinotecan is still unclear. Second, currently, there are no suitable large-sample clinical datasets that directly support our conclusions, and relevant clinical research needs to be further conducted to verify our hypothesis; moreover, we have initialed a clinical trial(NCT03162705) and hope this onging clincal trial could provide more direct evidence onni. Third, the accuracy of the model prediction is inadequate, and it may be necessary to expand the model to optimize it.

Conclusion

In conclusion, we analyzed the mutation and gene expression data from the GDSC of 54 ES-SCLC cell lines with regard to etoposide susceptibility and found that the panel including *CSMD3, EPB41L3, PCLO*, and *RYR1* can likely predict the sensitivity of ES-SCLC to etoposide and, therefore, the clinical survival of patients with SCLC.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Etoposide IC50 values of 54 SCLC cell lines

Cell line	IC50 (μM)	AUC
LU-135	0.242	0.262
SBC-3	0.276	0.292
SBC-5	0.406	0.344
LU-134-A	0.407	0.363
NCI-H526	0.515	0.393
NCI-H1048	0.563	0.405
DMS-273	0.595	0.42
NCI-H211	0.618	0.423
NCI-H187	0.758	0.458
NCI-H748	0.838	0.475
NCI-H209	0.97	0.495
IST-SL2	0.978	0.496
SW1271	1.29	0.537
COR-L279	1.39	0.555
NCI-H1694	1.52	0.566
LB647-SCLC	1.77	0.585
COLO-668	2.01	0.61
NCI-H1876	2.06	0.614
NCI-H1304	2.34	0.629
NCI-H1417	3.26	0.669
MS-1	3.62	0.709
NCI-H64	3.93	0.742
NCI-H2081	4.28	0.715
LU-139	4.7	0.71
NCI-H69	5.35	0.74
NCI-H1963	6.37	0.795
NCI-H510A	6.78	0.795
NCI-H847	7.38	0.827
NCI-H2141	7.39	0.797
NCI-H2196	8.08	0.798
IST-SL1	10.5	0.83
LU-165	10.9	0.821
NCI-H1688	11	0.825
NCI-H2029	12.3	0.867
NCI-H841	15.2	0.871
CPC-N	16.4	0.865
COR-L95	17.5	0.86
DMS-79	21.4	0.877
COR-L88	22	0.876
NCI-H2171	23.8	0.933
SBC-1	33.3	0.935
NCI-H82	36	0.942
NCI-H1836	41.1	0.928
NCI-H446	45.6	0.936
NCI-H524	50	0.965
SHP-77	57.7	0.97
NCI-H1092	65.2	0.96
NCI-H2227	69.3	0.949
DMS-53	71.3	0.955
HCC-33	73.8	0.964
NCI-H196	108	0.971
NCI-H1436	133	0.968
NCI-H345	162	0.978
DMS-114	319	0.984

(*Continued*)

Table S1 (Continued)

Abbreviations: AUC, area under the curve; IC50, half maximal inhibitory concentration; SCLC, small cell lung cancer.

Table S2 ROC curve of all genes (mutation frequency >10%)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CSMD3	0.697	0.077	0.016	0.546	0.848
USP34	0.685	0.099	0.053	0.49	0.879
MYO18B	0.679	0.096	0.061	0.491	0.867
ABCA13	0.673	0.093	0.07	0.491	0.855
DNAH2	0.673	0.099	0.07	0.479	0.866
LAMA5	0.661	0.099	0.092	0.468	0.854
SCN4A	0.655	0.101	0.105	0.457	0.853
ARAP2	0.643	0.101	0.134	0.446	0.84
CNTRL	0.643	0.101	0.134	0.446	0.84
ENSG00000250423	0.643	0.101	0.134	0.446	0.84
RYR1	0.631	0.082	0.111	0.469	0.792
EYS	0.631	0.096	0.17	0.443	0.818
HSPG2	0.631	0.1	0.17	0.435	0.827
NLRP5	0.631	0.1	0.17	0.435	0.827
UNC13C	0.631	0.1	0.17	0.435	0.827
DDX12	0.619	0.1	0.212	0.424	0.814
XIRP2	0.619	0.096	0.212	0.432	0.806
EPB41L3	0.61	0.083	0.179	0.447	0.774
COL3A1	0.607	0.099	0.261	0.413	0.802
NIPBL	0.607	0.099	0.261	0.413	0.802
NLRP3	0.607	0.099	0.261	0.413	0.802
POLQ	0.607	0.099	0.261	0.413	0.802
GRM5	0.601	0.101	0.289	0.404	0.798
PKD1L1	0.601	0.097	0.289	0.411	0.792
REG3G	0.601	0.101	0.289	0.404	0.798
AHNAK	0.595	0.099	0.318	0.402	0.789
PCLO	0.591	0.083	0.267	0.429	0.754
AC027369_8	0.589	0.1	0.349	0.393	0.785
BRIP1	0.589	0.1	0.349	0.393	0.785
COL6A3	0.589	0.1	0.349	0.393	0.785
ERBB4	0.589	0.1	0.349	0.393	0.785
FAM135B	0.589	0.097	0.349	0.399	0.779
FBN1	0.589	0.1	0.349	0.393	0.785
FREM1	0.589	0.1	0.349	0.393	0.785
HFM1	0.589	0.1	0.349	0.393	0.785
KDR	0.589	0.1	0.349	0.393	0.785
MYH1	0.589	0.1	0.349	0.393	0.785
NDST4	0.589	0.1	0.349	0.393	0.785
PPP1R9A	0.589	0.1	0.349	0.393	0.785
SMARCA4	0.589	0.1	0.349	0.393	0.785
THSD7B	0.589	0.1	0.349	0.393	0.785
UBQLN3	0.589	0.1	0.349	0.393	0.785
NAV3	0.583	0.098	0.382	0.391	0.776
ADAMTS16	0.577	0.099	0.417	0.383	0.772
AKAP13	0.577	0.099	0.417	0.383	0.772
ALPK2	0.577	0.099	0.417	0.383	0.772
COL14A1	0.577	0.099	0.417	0.383	0.772
DPP10	0.577	0.099	0.417	0.383	0.772
EML5	0.577	0.099	0.417	0.383	0.772
KIAA1109	0.577	0.099	0.417	0.383	0.772

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
LYST	0.577	0.099	0.417	0.383	0.772
MYH13	0.577	0.099	0.417	0.383	0.772
MYH7	0.577	0.099	0.417	0.383	0.772
PDGFRA	0.577	0.099	0.417	0.383	0.772
ZEB1	0.577	0.099	0.417	0.383	0.772
LRRK2	0.571	0.098	0.454	0.38	0.763
ACAN	0.565	0.099	0.492	0.372	0.759
ADAMTSL1	0.565	0.099	0.492	0.372	0.759
ADCY8	0.565	0.099	0.492	0.372	0.759
ALMS1	0.565	0.099	0.492	0.372	0.759
ANKS1B	0.565	0.099	0.492	0.372	0.759
CNTNAP4	0.565	0.099	0.492	0.372	0.759
FRAS1	0.565	0.099	0.492	0.372	0.759
LAMA1	0.565	0.099	0.492	0.372	0.759
MORC1	0.565	0.099	0.492	0.372	0.759
MUC16	0.565	0.092	0.492	0.385	0.746
MUC5B	0.565	0.097	0.492	0.376	0.755
PTPRB	0.565	0.099	0.492	0.372	0.759
SIGLEC10	0.565	0.099	0.492	0.372	0.759
STAB2	0.565	0.099	0.492	0.372	0.759
SYNE1	0.565	0.097	0.492	0.376	0.755
UBR4	0.565	0.099	0.492	0.372	0.759
DNAH8	0.56	0.097	0.533	0.368	0.751
RELN	0.56	0.097	0.533	0.368	0.751
TP53	0.56	0.089	0.533	0.385	0.734
WDR72	0.56	0.099	0.533	0.365	0.754
ZNF831	0.56	0.099	0.533	0.365	0.754
ADAMTS12	0.554	0.098	0.574	0.361	0.746
ADGB	0.554	0.098	0.574	0.361	0.746
FBN2	0.554	0.098	0.574	0.361	0.746
GPR112	0.554	0.098	0.574	0.361	0.746
ITGAD	0.554	0.098	0.574	0.361	0.746
KALRN	0.554	0.098	0.574	0.361	0.746
KIF2B	0.554	0.098	0.574	0.361	0.746
PKHD1L1	0.554	0.098	0.574	0.361	0.746
TG	0.554	0.098	0.574	0.361	0.746
WDR87	0.554	0.098	0.574	0.361	0.746
ANKRD11	0.548	0.099	0.618	0.354	0.741
CNTN5	0.548	0.099	0.618	0.354	0.741
COL12A1	0.548	0.097	0.618	0.357	0.738
COL17A1	0.548	0.099	0.618	0.354	0.741
CPS1	0.548	0.099	0.618	0.354	0.741
DAPK1	0.548	0.099	0.618	0.354	0.741
DNAH6	0.548	0.099	0.618	0.354	0.741
FCGBP	0.548	0.097	0.618	0.357	0.738
GLI3	0.548	0.099	0.618	0.354	0.741
GRIN2B	0.548	0.099	0.618	0.354	0.741
HECW1	0.548	0.099	0.618	0.354	0.741
HYDIN	0.548	0.095	0.618	0.361	0.735

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
IGSF3	0.548	0.099	0.618	0.354	0.741
KIAA1409	0.548	0.099	0.618	0.354	0.741
LINGO2	0.548	0.099	0.618	0.354	0.741
LRRIQ1	0.548	0.099	0.618	0.354	0.741
MADD	0.548	0.099	0.618	0.354	0.741
MCF2	0.548	0.099	0.618	0.354	0.741
PLXNA4	0.548	0.099	0.618	0.354	0.741
RYR2	0.548	0.095	0.618	0.361	0.735
SORCS3	0.548	0.099	0.618	0.354	0.741
UNC80	0.548	0.097	0.618	0.357	0.738
WDR17	0.548	0.099	0.618	0.354	0.741
CUBN	0.542	0.098	0.662	0.351	0.733
DSCAML1	0.542	0.098	0.662	0.351	0.733
ENSG00000121031	0.542	0.098	0.662	0.351	0.733
ENSG00000188219	0.542	0.098	0.662	0.351	0.733
FAT3	0.542	0.096	0.662	0.353	0.73
LAMA2	0.542	0.098	0.662	0.351	0.733
SYNE2	0.542	0.098	0.662	0.351	0.733
TAF1L	0.542	0.098	0.662	0.351	0.733
TNN	0.542	0.098	0.662	0.351	0.733
ZNF99	0.542	0.098	0.662	0.351	0.733
ACSM2B	0.536	0.098	0.708	0.344	0.727
ASPM	0.536	0.098	0.708	0.344	0.727
ATP10D	0.536	0.098	0.708	0.344	0.727
BCLAF1	0.536	0.098	0.708	0.344	0.727
C12orf35	0.536	0.098	0.708	0.344	0.727
C6	0.536	0.098	0.708	0.344	0.727
CACNA1H	0.536	0.098	0.708	0.344	0.727
CDH19	0.536	0.098	0.708	0.344	0.727
COL19A1	0.536	0.098	0.708	0.344	0.727
COL24A1	0.536	0.098	0.708	0.344	0.727
CREBBP	0.536	0.098	0.708	0.344	0.727
DCHS2	0.536	0.098	0.708	0.344	0.727
DNAH17	0.536	0.098	0.708	0.344	0.727
DOCK7	0.536	0.098	0.708	0.344	0.727
EP400	0.536	0.098	0.708	0.344	0.727
IGF2R	0.536	0.098	0.708	0.344	0.727
LTBP1	0.536	0.098	0.708	0.344	0.727
MUC17	0.536	0.097	0.708	0.346	0.725
MYH11	0.536	0.098	0.708	0.344	0.727
NOTCH1	0.536	0.098	0.708	0.344	0.727
OTOF	0.536	0.098	0.708	0.344	0.727
PIK3CG	0.536	0.098	0.708	0.344	0.727
POM121L12	0.536	0.098	0.708	0.344	0.727
POTEC	0.536	0.098	0.708	0.344	0.727
POTEG	0.536	0.098	0.708	0.344	0.727
PTEN	0.536	0.098	0.708	0.344	0.727
ROBO4	0.536	0.098	0.708	0.344	0.727
SCN1A	0.536	0.098	0.708	0.344	0.727

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
SLC5A10	0.536	0.098	0.708	0.344	0.727
SLIT3	0.536	0.098	0.708	0.344	0.727
SRCAP	0.536	0.098	0.708	0.344	0.727
TRHDE	0.536	0.098	0.708	0.344	0.727
TTN	0.536	0.093	0.708	0.354	0.718
VWA3B	0.536	0.098	0.708	0.344	0.727
WBSCR17	0.536	0.098	0.708	0.344	0.727
WNK3	0.536	0.098	0.708	0.344	0.727
ZNF208	0.536	0.098	0.708	0.344	0.727
ZNF804B	0.536	0.098	0.708	0.344	0.727
ZSCAN20	0.536	0.098	0.708	0.344	0.727
DOCK11	0.53	0.098	0.755	0.338	0.722
PKHD1	0.53	0.097	0.755	0.34	0.72
SPTA1	0.53	0.097	0.755	0.34	0.72
ZFHX4	0.53	0.096	0.755	0.342	0.718
ZNF536	0.53	0.097	0.755	0.34	0.72
ABCA12	0.524	0.097	0.803	0.334	0.714
ABCB1	0.524	0.097	0.803	0.334	0.714
AC007731.1	0.524	0.097	0.803	0.334	0.714
ANKRD30B	0.524	0.097	0.803	0.334	0.714
C20orf26	0.524	0.097	0.803	0.334	0.714
C7orf58	0.524	0.097	0.803	0.334	0.714
CACNA1C	0.524	0.097	0.803	0.334	0.714
DMD	0.524	0.097	0.803	0.334	0.714
DPP6	0.524	0.097	0.803	0.334	0.714
FLG2	0.524	0.097	0.803	0.334	0.714
GRM1	0.524	0.097	0.803	0.334	0.714
HMCN1	0.524	0.096	0.803	0.335	0.712
MAGEC1	0.524	0.097	0.803	0.334	0.714
MDN1	0.524	0.097	0.803	0.334	0.714
MGAM	0.524	0.097	0.803	0.334	0.714
MKI67	0.524	0.097	0.803	0.334	0.714
MUC12	0.524	0.096	0.803	0.335	0.712
MUC2	0.524	0.097	0.803	0.334	0.714
NID2	0.524	0.097	0.803	0.334	0.714
OR8K1	0.524	0.097	0.803	0.334	0.714
PAPPA	0.524	0.097	0.803	0.334	0.714
PTPN13	0.524	0.097	0.803	0.334	0.714
SAMD9	0.524	0.097	0.803	0.334	0.714
SI	0.524	0.097	0.803	0.334	0.714
SPHKAP	0.524	0.096	0.803	0.335	0.712
TPO	0.524	0.097	0.803	0.334	0.714
USP32	0.524	0.097	0.803	0.334	0.714
VCAN	0.524	0.097	0.803	0.334	0.714
WRN	0.524	0.097	0.803	0.334	0.714
ZEB2	0.524	0.097	0.803	0.334	0.714
ZNF479	0.524	0.097	0.803	0.334	0.714
DNAH11	0.518	0.096	0.851	0.329	0.707
DNAH14	0.518	0.096	0.851	0.329	0.707

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
GABRA5	0.518	0.097	0.851	0.328	0.708
VPS13B	0.518	0.096	0.851	0.329	0.707
ABCC11	0.512	0.096	0.901	0.323	0.7
CCDC141	0.512	0.096	0.901	0.323	0.7
CDH10	0.512	0.096	0.901	0.323	0.7
CDH8	0.512	0.096	0.901	0.323	0.7
CEP350	0.512	0.096	0.901	0.323	0.7
COL11A2	0.512	0.096	0.901	0.323	0.7
CRB1	0.512	0.096	0.901	0.323	0.7
DOCK2	0.512	0.096	0.901	0.323	0.7
LAMA3	0.512	0.096	0.901	0.323	0.7
POTEH	0.512	0.096	0.901	0.323	0.7
PXDNL	0.512	0.096	0.901	0.323	0.7
SAMD9L	0.512	0.096	0.901	0.323	0.7
SPAG17	0.512	0.096	0.901	0.323	0.7
TPTE	0.512	0.096	0.901	0.323	0.7
CACNA1E	0.506	0.096	0.95	0.318	0.694
FAM5B	0.506	0.096	0.95	0.318	0.694
FAT4	0.506	0.096	0.95	0.318	0.693
HRNR	0.506	0.096	0.95	0.318	0.693
MDGA2	0.506	0.096	0.95	0.318	0.694
MYCBP2	0.506	0.096	0.95	0.318	0.694
NBPF10	0.506	0.096	0.95	0.318	0.693
OR10J1	0.506	0.096	0.95	0.318	0.694
TNXB	0.506	0.096	0.95	0.318	0.693
TRPA1	0.506	0.096	0.95	0.318	0.694
ZIC1	0.506	0.096	0.95	0.318	0.694
ABCA9	0.5	0.095	1	0.313	0.687
DNAH3	0.5	0.095	1	0.313	0.687
FAM75D4	0.5	0.095	1	0.313	0.687
FMN2	0.5	0.095	1	0.313	0.687
KIAA0947	0.5	0.095	1	0.313	0.687
MTUS2	0.5	0.095	1	0.313	0.687
MYH4	0.5	0.095	1	0.313	0.687
NEB	0.5	0.095	1	0.313	0.687
OR14K1	0.5	0.095	1	0.313	0.687
SLC8A3	0.5	0.095	1	0.313	0.687
TEP1	0.5	0.095	1	0.313	0.687
THSD7A	0.5	0.095	1	0.313	0.687
USH2A	0.5	0.095	1	0.313	0.687
C15orf2	0.494	0.095	0.95	0.308	0.68
CDH20	0.494	0.095	0.95	0.308	0.68
COL11A1	0.494	0.095	0.95	0.308	0.68
COL5A2	0.494	0.095	0.95	0.308	0.68
DNAH9	0.494	0.095	0.95	0.308	0.68
FSTL5	0.494	0.095	0.95	0.308	0.68
GRIP1	0.494	0.095	0.95	0.308	0.68
KIF21A	0.494	0.095	0.95	0.308	0.68
MYO7A	0.494	0.095	0.95	0.308	0.68

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
MYPN	0.494	0.095	0.95	0.308	0.68
NALCN	0.494	0.095	0.95	0.308	0.68
PHKB	0.494	0.095	0.95	0.308	0.68
PRUNE2	0.494	0.095	0.95	0.308	0.68
SCN7A	0.494	0.095	0.95	0.308	0.68
SPEG	0.494	0.095	0.95	0.308	0.68
TFAP2D	0.494	0.095	0.95	0.308	0.68
ZFPM2	0.494	0.095	0.95	0.308	0.68
ZNF142	0.494	0.095	0.95	0.308	0.68
AHNAK2	0.488	0.095	0.901	0.303	0.673
DNAH7	0.488	0.095	0.901	0.303	0.673
HCN1	0.488	0.095	0.901	0.303	0.673
PCDH15	0.488	0.095	0.901	0.303	0.673
ZNF729	0.488	0.095	0.901	0.303	0.673
BSN	0.482	0.094	0.851	0.298	0.666
CENPF	0.482	0.094	0.851	0.298	0.666
CLSTN2	0.482	0.094	0.851	0.298	0.666
FLNC	0.482	0.094	0.851	0.298	0.666
HEATR1	0.482	0.094	0.851	0.298	0.666
KIAA1239	0.482	0.094	0.851	0.298	0.666
LCT	0.482	0.094	0.851	0.298	0.666
LPHN3	0.482	0.094	0.851	0.298	0.666
MLL2	0.482	0.094	0.851	0.297	0.667
ODZ2	0.482	0.094	0.851	0.298	0.666
OR5T2	0.482	0.094	0.851	0.298	0.666
OR6Y1	0.482	0.094	0.851	0.298	0.666
PCDH11X	0.482	0.094	0.851	0.298	0.666
PCDHB7	0.482	0.094	0.851	0.298	0.666
PKD1L2	0.482	0.094	0.851	0.298	0.666
PLCH1	0.482	0.094	0.851	0.298	0.666
PTPRD	0.482	0.094	0.851	0.298	0.666
RGPD3	0.482	0.094	0.851	0.298	0.666
SELP	0.482	0.094	0.851	0.298	0.666
SYTL2	0.482	0.094	0.851	0.298	0.666
TKTL2	0.482	0.094	0.851	0.298	0.666
TYR	0.482	0.094	0.851	0.298	0.666
UTP20	0.482	0.094	0.851	0.298	0.666
VWF	0.482	0.094	0.851	0.298	0.666
APOB	0.476	0.094	0.803	0.293	0.66
CNTNAP5	0.476	0.094	0.803	0.293	0.66
EP300	0.476	0.094	0.803	0.293	0.66
HEATR7B2	0.476	0.094	0.803	0.293	0.66
ROS1	0.476	0.094	0.803	0.293	0.66
ZIM2	0.476	0.094	0.803	0.293	0.66
ABCA8	0.47	0.093	0.755	0.288	0.652
ABCC12	0.47	0.093	0.755	0.288	0.652
ACSM5	0.47	0.093	0.755	0.288	0.652
ADAM2	0.47	0.093	0.755	0.288	0.652
ANKRD55	0.47	0.093	0.755	0.288	0.652

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
ATP1A2	0.47	0.093	0.755	0.288	0.652
C10orf112	0.47	0.093	0.755	0.288	0.652
C12orf51	0.47	0.093	0.755	0.288	0.652
CMYA5	0.47	0.093	0.755	0.288	0.652
CSMD1	0.47	0.094	0.755	0.286	0.654
CYP11B1	0.47	0.093	0.755	0.288	0.652
DCHS1	0.47	0.093	0.755	0.288	0.652
DSEL	0.47	0.093	0.755	0.288	0.652
DYSF	0.47	0.093	0.755	0.288	0.652
FAT1	0.47	0.093	0.755	0.288	0.652
HERC2	0.47	0.093	0.755	0.288	0.652
KCNU1	0.47	0.093	0.755	0.288	0.652
LRP1B	0.47	0.095	0.755	0.284	0.656
MSH4	0.47	0.093	0.755	0.288	0.652
MYH15	0.47	0.093	0.755	0.288	0.652
MYH2	0.47	0.093	0.755	0.288	0.652
MYO9A	0.47	0.093	0.755	0.288	0.652
NLRP4	0.47	0.093	0.755	0.288	0.652
OBSCN	0.47	0.094	0.755	0.286	0.654
PRDM9	0.47	0.093	0.755	0.288	0.652
PTPRU	0.47	0.093	0.755	0.288	0.652
SZT2	0.47	0.093	0.755	0.288	0.652
TNR	0.47	0.093	0.755	0.288	0.652
TRPM2	0.47	0.093	0.755	0.288	0.652
UTRN	0.47	0.093	0.755	0.288	0.652
ZNF462	0.47	0.093	0.755	0.288	0.652
ZNF534	0.47	0.093	0.755	0.288	0.652
ANK2	0.464	0.093	0.708	0.282	0.646
COL22A1	0.464	0.093	0.708	0.282	0.646
DST	0.464	0.093	0.708	0.282	0.646
GRIN2A	0.464	0.092	0.708	0.285	0.644
RYR3	0.464	0.093	0.708	0.282	0.646
SLCO1B1	0.464	0.092	0.708	0.285	0.644
ABCB5	0.458	0.092	0.662	0.279	0.638
BAI3	0.458	0.092	0.662	0.279	0.638
C5orf42	0.458	0.092	0.662	0.279	0.638
CD163	0.458	0.092	0.662	0.279	0.638
DCC	0.458	0.092	0.662	0.279	0.638
MYO7B	0.458	0.092	0.662	0.279	0.638
NLRP12	0.458	0.092	0.662	0.279	0.638
ODZ1	0.458	0.092	0.662	0.279	0.638
ODZ3	0.458	0.092	0.662	0.279	0.638
OR8H3	0.458	0.092	0.662	0.279	0.638
PDE4DIP	0.458	0.092	0.662	0.279	0.638
RIMS2	0.458	0.092	0.662	0.279	0.638
SACS	0.458	0.092	0.662	0.279	0.638
SVEP1	0.458	0.092	0.662	0.279	0.638
TCHH	0.458	0.092	0.662	0.279	0.638
ZNF521	0.458	0.092	0.662	0.279	0.638

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
C1orf173	0.452	0.092	0.618	0.272	0.633
DOCK4	0.452	0.09	0.618	0.275	0.629
GPR98	0.452	0.092	0.618	0.272	0.633
KIAA1549	0.452	0.09	0.618	0.275	0.629
MACF1	0.452	0.092	0.618	0.272	0.633
CDH18	0.446	0.091	0.574	0.269	0.624
CTNNA2	0.446	0.091	0.574	0.269	0.624
DNAH5	0.446	0.091	0.574	0.269	0.624
FAM5C	0.446	0.091	0.574	0.269	0.624
TRRAP	0.446	0.091	0.574	0.269	0.624
BRWD3	0.44	0.089	0.533	0.266	0.615
CACHD1	0.44	0.089	0.533	0.266	0.615
CDH7	0.44	0.089	0.533	0.266	0.615
DSCAM	0.44	0.089	0.533	0.266	0.615
LRP2	0.44	0.091	0.533	0.262	0.619
MUC19	0.44	0.091	0.533	0.262	0.619
OR11H12	0.44	0.089	0.533	0.266	0.615
OR52R1	0.44	0.089	0.533	0.266	0.615
SIGLEC8	0.44	0.089	0.533	0.266	0.615
TMEM132D	0.44	0.091	0.533	0.262	0.619
MUC4	0.435	0.094	0.492	0.25	0.619
AIM1	0.429	0.088	0.454	0.257	0.6
CARD11	0.429	0.088	0.454	0.257	0.6
COL5A3	0.429	0.088	0.454	0.257	0.6
CSMD2	0.429	0.088	0.454	0.257	0.6
EYA4	0.429	0.088	0.454	0.257	0.6
FREM3	0.429	0.088	0.454	0.257	0.6
KIAA0240	0.429	0.088	0.454	0.257	0.6
KIAA1211	0.429	0.088	0.454	0.257	0.6
LAMC3	0.429	0.088	0.454	0.257	0.6
LPA	0.429	0.088	0.454	0.257	0.6
LRFN5	0.429	0.088	0.454	0.257	0.6
NAV2	0.429	0.088	0.454	0.257	0.6
NCAM2	0.429	0.088	0.454	0.257	0.6
SDK1	0.429	0.088	0.454	0.257	0.6
SETD2	0.429	0.088	0.454	0.257	0.6
SHROOM3	0.429	0.088	0.454	0.257	0.6
SPTB	0.429	0.088	0.454	0.257	0.6
ANKRD30A	0.423	0.089	0.417	0.249	0.596
OTOG	0.423	0.089	0.417	0.249	0.596
PAPPA2	0.423	0.089	0.417	0.249	0.596
C10orf71	0.417	0.086	0.382	0.247	0.586
COL6A6	0.417	0.086	0.382	0.247	0.586
FLG	0.417	0.09	0.382	0.241	0.592
FSCB	0.417	0.086	0.382	0.247	0.586
PCNX	0.417	0.086	0.382	0.247	0.586
XDH	0.417	0.086	0.382	0.247	0.586
BOD1L	0.405	0.085	0.318	0.238	0.571
LRRC7	0.405	0.085	0.318	0.238	0.571

(*Continued*)

Table S2 (Continued)

Test result variable(s)	Area	Standard error[a]	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
RP1L1	0.405	0.085	0.318	0.238	0.571
ADAMTS20	0.399	0.086	0.289	0.23	0.568
MLL3	0.393	0.084	0.261	0.229	0.557
DNAH10	0.369	0.081	0.17	0.21	0.528
RB1	0.369	0.096	0.17	0.182	0.557

Note: [a]Under the nonparametric assumption.
Abbreviation: ROC, receiver operating characteristic.