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Adenosine triphosphate-binding cassette subfamily C members in liver hepatocellular carcinoma

Bioinformatics-driven prognostic value

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Abstract

Aberrant expression of adenosine triphosphate-binding cassette subfamily C (ABCC), one of the largest superfamilies and transporter gene families of membrane proteins, is associated with various tumors. However, its relationship with liver hepatocellular carcinoma (LIHC) remains unclear.

We used the Oncomine, UALCAN, Human Protein Atlas, GeneMANIA, GO, Kyoto Encyclopedia of Genes and Genomes (KEGG), TIMER, and Kaplan–Meier Plotter databases. On May 20, 2021, we searched these databases for the terms ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, ABCC7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, ABCC13, and "liver cancer." The exposure group comprised LIHC patients, and the control group comprised normal patients (those with noncancerous liver tissue). All patients shown in the retrieval language search were included. We compared the mRNA expression of these proteins in LIHC and control patients to examine the potential role of ABCC1–13 in LIHC.

Relative to the normal liver tissue, mRNA expression of ABCC1/2/3/4/5/6/10 was significantly upregulated ($P < .001$), and that of ABCC9/11 significantly downregulated (both $P < .001$), in LIHC. ABCC mRNA expression varied with gender ($P < .05$), except for ABCC11–13; with tumor grade ($P < 0.05$), except for ABCC7/12/13; with tumor stage ($P < .05$), except for ABCC11–13; and with lymph node metastasis status ($P < .05$), except for ABCC7/8/11/12/13. Based on KEGG enrichment analysis, these genes were associated with the following pathways: ABC transporters, Bile secretion, Antifolate resistance, and Peroxisome ($P < .05$). Except for ABCC12/13, the ABCCs were significantly associated with B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil, and dendritic cell infiltration ($P < .05$). High mRNA expression of ABCC1/4/5/8 ($P < .05$) and low expression of ABCC6/7/9/12/13 ($P < .05$) indicated poor prognosis. Prognostic significance was indicated for ABCC2/13 for both men and women ($P < .05$); for ABCC1/6/12/13 for tumor grades 1–3 ($P < .05$); for ABCC5/11/12/13 for all tumor stages ($P < .05$); for ABCC1/11/12/13 for American Joint Committee on Cancer T stages 1–3 ($P < .05$); and for ABCC1/5/6/13 for vascular invasion. None showed prognostic significance for microvascular invasion ($P < .05$).

We identified ABCC1/2/3/4/5/6/9/10/11 as potential diagnostic markers, and ABCC1/4/5/6/7/8/9/12/13 as prognostic markers, of LIHC. Our future work will promote the use of ABCCs in the diagnosis and treatment of LIHC.

Abbreviations: ABCC = adenosine triphosphate-binding cassette subfamily C, AJCC = American Joint Committee on Cancer, GO = gene ontology database, HPA = human protein atlas, KEGG = kyoto encyclopedia of genes and genomes, LIHC = liver hepatocellular carcinoma, OS = overall survival.

Keywords: ABCC family, bioinformatics, diagnostic marker, liver hepatocellular carcinoma, prognostic marker

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SD and MX contributed equally to this work.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Liver hepatocellular carcinoma (LIHC), the fourth leading cause of cancer-related deaths worldwide, accounts for 90% of primary liver cancers. In 2018, it caused ca. 625,000 deaths worldwide.[1,2] LIHC is caused by factors such as hepatitis B, hepatitis C virus infection, and aflatoxin exposure. Its onset is insidious, with no obvious symptoms; it is usually discovered in the middle and late stages, when the best opportunity for surgical resection, the most frequently recommend treatment, has already passed. Early detection is therefore important. Other current typical treatments include antiviral therapy, liver transplantation, and chemotherapy. In spite of the available treatments, prognosis remains poor,[3] and screening of novel LIHC biomarkers is required to improve early diagnosis and prognosis.[4] AFP performs poorly as a marker for LIHC detection, increasing the rate of missed diagnoses. Molecular targeted therapy and immunotherapy for LIHC have emerged as research hotspots. For example, therapies targeting Bak1, TAA, and other proteins have therapeutic effects on LIHC, but need to be improved.[5,6]

The adenosine triphosphate-binding cassette subfamily C (ABCC) superfamily is another promising target, Its aberrant expression is associated with various tumors.[10–14] It consists of 48 ABCC transporters, being one of the largest superfamilies of membrane proteins in prokaryotes and eukaryotes.[7] It is also the largest transporter gene family: the members bind with ATP and use this energy to drive the transport of sugars, metal ions, compounds, and other molecules.[8] There are 7 subfamilies, ABCA, ABCB, ABCC (ABCC1–13),[9] ABCD, ABCE, ABCF, and ABCG. ABCC1 (also known as MRP1) can promote the excretion of heterogeneous and endogenous organic anions, and confer multidrug resistance via the efflux of active drugs, thus protecting human organs and tissues from cytotoxicity. ABCC1 is associated with progression and drug resistance in various cancers, including LIHC, prostate cancer, and colon cancer.[15–18]

ABCC2 (MRP2) plays an important role in the transportation of endogenous and exogenous substances, as well as drug absorption, distribution, and excretion, and is associated with colorectal cancer, renal cell carcinoma, multiple myeloma, and other tumors.[19–22] ABCC3 (MRP3), which is responsible for binding, hydrolysis, and ATP release during molecular transport, is vital in the transport and regulation of different organic and toxic compounds. It has great potential for improving cancer treatment and survival.[23] ABCC4 (MRP4), which can transport various organic anionic compounds out of cells, is widely used as a drug transporter in tumors, and is associated with colon cancer, pancreatic cancer, and esophageal squamous cell carcinoma.[24–26] ABCC5 (MRP5), is an organic anion transporter with excellent ability to transport nucleotides and nucleotide analogs, and is associated with breast cancer bone metastasis and prostate cancer progression.[27–29] ABCC6 is an ATP-dependent transmembrane transporter, mainly expressed in the liver and kidney, and is a potential target for tumor treatment.[30,31] ABCC7 (CFTR), mainly expressed in colon tissue and skin, regulates ion and liquid transport in epithelial tissues.[32] ABCC8 and ABCC9 are indispensable to the KATP channel, and are closely associated with neonatal diabetes, pulmonary hypertension, and other diseases.[33–35] ABCC10 (MRP7), which plays a role in drug resistance, transports various chemotherapeutic drugs, including taxanes, epothilone B, and vinca alkaloids.[36] ABCC11 (MRP8) is associated with the risk of breast cancer.[37] A harmful mutation of ABCC12 (MRP9) can cause cholestasis.[38] ABCC13 (MRP10), a pseudogene, is highly expressed in human fetal liver.[39,40]

Nonetheless, little is known about the role of ABCCs in LIHC. We therefore examined their expression and prognostic value in LIHC patients, via a retrospective bioinformatics-driven approach.

2. Materials and methods

2.1. Oncomine database

We used the online Oncomine database (http://www.oncomine.org), a cancer microarray database for genome-wide expression analysis, to analyze ABCC mRNA expression in various cancers. Oncomine includes 715 datasets and 86733 samples, covering 35 cancer types.[41] We determined statistical difference via the Student *t* test, and determined differences in mRNA expression based on $P < .0001$, fold change = 1.5, and gene grade = 10%.

2.2. UALCAN database

UALCAN (http://ualcan.path.uab.edu) is an online database that uses data from The Cancer Genome Atlas database, and contains RNA-seq data for 31 cancer types.[42] We performed a genome-wide analysis of ABCC expression in LIHC, using UALCAN data for 371 LIHC patients and 50 normal controls (patients with noncancerous liver tissue), accounting for gender, tumor grade, tumor stage, and lymph node metastasis. UALCAN provides all statistically significant results ($P < .05$). We excluded records with transcripts per million (TPM) < 1.

2.3. Human protein atlas

From the Human Protein Atlas (HPA) (http://www.proteinatlas.org), which collects representative immunohistochemistry-based protein expression data for nearly 20 highly common cancers,[43] we obtained immunohistochemical images of ABCC protein expression in clinical specimens from patients with LIHC and normal tissues. We selected HPA records with $P < .05$.

2.4. GeneMANIA database

Using GeneMANIA (http://www.genemania.org), an online tool that uses available genomics and proteomics data to generate hypotheses involving gene function,[44] we analyzed the functional association network between ABCC family members and their related genes. The advanced statistics option is a maximum synthetic attribute of 10 and a maximum synthetic gene of 20. GeneMANIA considers $P < .05$ statistically significant.

2.5. Gene ontology (GO) and kyoto encyclopedia of genes and genomes pathway enrichment

The GO database (http://geneontology.org) comprehensively describes the attributes of genes and gene products in an organism in terms of the molecular function of the genes, the function of cell components, and the biological processes involved.[45] KEGG (http://www.kegg.jp) integrates information on genome, chemistry, and system function.[46] We used the Bioconductor plugin in R for GO and KEGG enrichment analysis, and considered $P < .05$ statistically significant.

2.6. TIMER database

The TIMER (https://cistrome.shinyapps.io/timer) database uses systematic analysis of microarray expression data to detect immune-cell penetration in tumor tissues, and to determine its association with various cancers, or with gene expression. We quantitatively analyzed the penetration ratios of 6 types of immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells).[47] We used TIMER to evaluate the immune infiltration of ABCC family members in LIHC, and analyzed the Spearman correlation between these 6 types of immune cells and ABCC mRNA expression. Statistical significance was set at $P < .05$.

2.7. Kaplan-meier plotter

Kaplan–Meier plotter (http://kmplot.com/analysis) is an online database for prognostic analysis of various types of cancer. It is based on the data sets of 3 major medical centers, in Berlin, Bethesda, and Melbourne.[48–51] For 364 LIHC patients, we evaluated overall survival (OS), determined the prognostic significance, and accounted for gender, tumor grade, tumor stage, American Joint Committee on Cancer (AJCC) T stage, and vascular invasion, with 95% confidence intervals and logarithmic *P* values. We used an OS chart to compare OS in the high- and low-expression groups. $P < .05$ was considered statistically significant. The probe numbers used to study ABCC1–13 were, respectively, 202804-at, 206155-at, 214979-at, 203196-at, 22636363-at, 214033-at, 205043-at, 210245-at, 208561-at, 213485-s-at, 224146-s-at, 1552590-at and 1552582-at.

2.8. Ethical approval

These analyses were based on online open-access databases, hence this article does not contain any research conducted by any author on human participants or animals, nor can it be followed up and updated.

2.9. Statistical analysis

SPSS 25.0 (IBM, Armonk, NY) was used for statistical analysis. Results were considered significant at $P < .05$. For Cox proportional hazard regression analysis, the 95% confidence interval (CI) and hazard ratio (HR) were used for risk assessment.

2.10. Data management and collection

We obtained records from the Oncomine, UALCAN, HPA, TIMER, and Kaplan–Meier plotter databases on May 20, 2021. Records were obtained by searching these databases using the terms ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, ABCC7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, ABCC13 and "liver cancer". The exposure group comprised patients with LIHC, and the control group comprised normal (noncancerous) tissue samples. All patients included in the search language search were included. The search was not restricted based on race, country, gender, or language. Two researchers (SD and MXT) independently reviewed the eligibility of the data, and XZ resolved any discrepancies. Disagreements over eligibility were resolved via discussion. The research selection process conformed to the STROBE guidelines. To ensure the validity and reliability of the results, SD and WS independently conducted statistical analysis. LYY reviewed the data to detect potential bias that could arise during subgroup analysis.

3. Results

3.1. mRNA expression of ABCCs in LIHC patients

Using the Oncomine database, we compared ABCC transcription in 20 cancers and normal tissues: mRNA expression of members ABCC1/4/5/6/7/10 was significantly higher, whereas that of ABCC9 was significantly lower, in LIHC tissue ($P < .05$). In the Roessler Liver 2 dataset,[52] the mRNA expression of ABCC6/7 was lower in LIHC tissue than in normal tissue (Fig. 1, Table 1).

We then verified these results using the UALCAN database. Relative to normal liver tissue, mRNA expression was upregulated for ABCC1/2/3/4/5/10 ($P < .0001$) and ABCC6 ($P < .001$) (Fig. 2), and downregulated in ABCC9/11 ($P < 0.0001$). ABCC8/12/13 were excluded from all analyses, because they had TPM < 1.

ABCC protein expression in LIHC was evaluated using the HPA database: that of ABCC2 and ABCC12 was downregulated, and that of ABCC3/4/8/9 was upregulated (Fig. 3).

3.1.1. ABCC mRNA expression in LIHC by gender. We compared 50 patients with normal (noncancerous) liver tissue, 245 male LIHC patients, and 117 female LIHC patients: except for ABCC2 and ABCC7, ABCC mRNA expression differed significantly between men and women (Fig. 4). Relative to normal liver tissue, mRNA expression in LIHC was significantly upregulated for ABCC1/3/4/5/6/10 in both men and women (Fig. 4A, C, D, E, F, J; $P < .0001$); that of ABCC9 was significantly downregulated in men ($P < .05$) and women ($P < .01$) (Fig. 4I); that of ABCC2 was upregulated in men (Fig. 4B, $P < .0001$); and that of ABCC7 was downregulated in women (Fig. 4G, $P < .05$).

3.1.2. ABCC mRNA expression in LIHC by tumor grade. We compared mRNA expression in 50 patients with normal (noncancerous) liver tissue, 54 grade 1 LIHC patients, 173 grade 2 patients, 118 grade 3 patients, and 12 grade 4 patients: members ABCC4/5/10 were highly upregulated in all grades (Fig. 5D, E, J; $P < .01$). For the other ABCC members (excluding ABCC7, Fig. 5G), mRNA expression did not differ significantly between the control and LIHC samples. LIHC tumor grade was significantly correlated with ABCC, for all ABCC members (Fig. 5A–F, H–K; $P < .05$).

3.1.3. ABCC mRNA expression in LIHC patients by tumor stage. We compared 50 patients with normal liver tissue with 168 stage 1, 84 stage 2, 82 stage 3, and 6 stage 4 LIHC patients: for all stages, mRNA expression was upregulated for ABCC4 ($P < .05$, Fig. 6D), but was not significantly different for ABCC8/11/12/13 (Fig. 6H, K, L, M). For the remaining ABCC members, mRNA expression was correlated with stage (Fig. 6A-G, I, J; $P < .05$).

3.1.4. ABCC mRNA expression in LIHC by lymph node metastasis status. We compared 50 patients with normal liver tissue, with 252 lymph node metastasis status N0 and 4 N1 status LIHC patients (Fig. 7). mRNA expression was highly upregulated for ABCC4 and ABCC5 for both N0 and N1 (Fig. 7D, E; $P < .05$); that of ABCC9 was significantly downregulated (Fig. 7I, $P < .001$); that of ABCC7/11 was not associated with lymphatic node metastasis status (Fig. 7G, K; $P \geq .05$); and that of ABCC1/2/3/6/10 was associated with lymph node metastasis (Fig. 7A, B, C, F, J; $P < .05$).

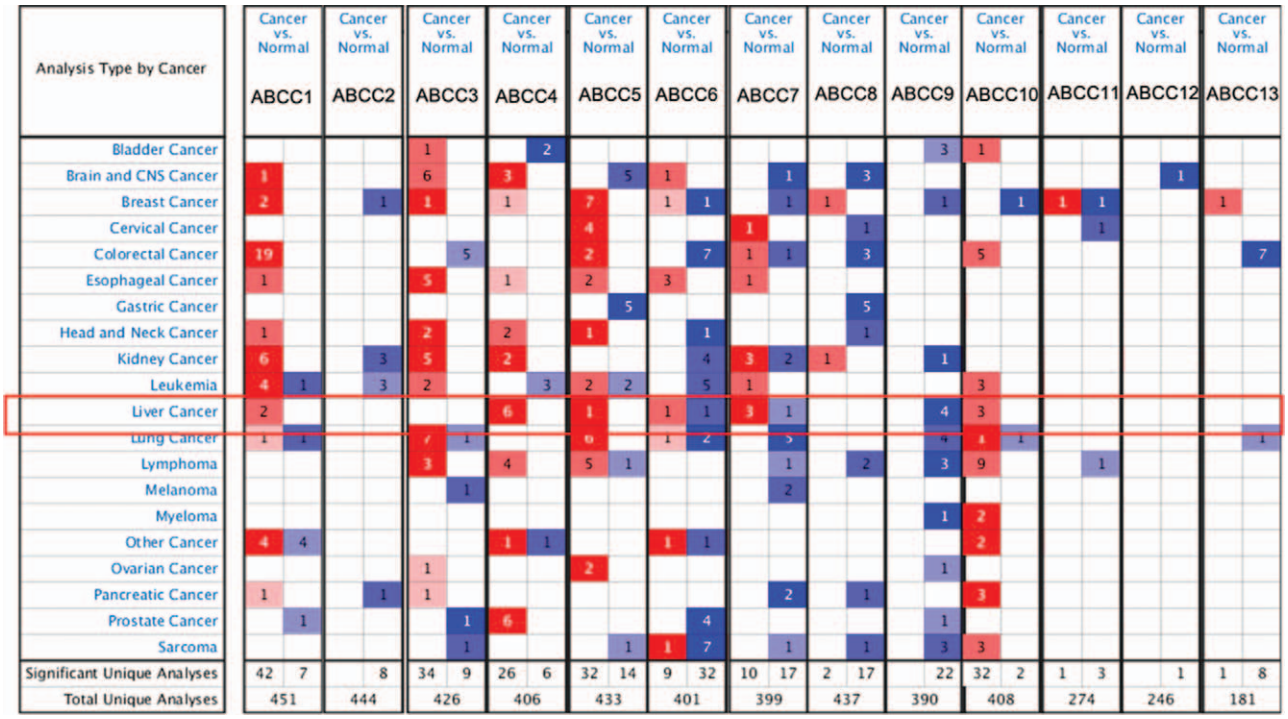

Figure 1. Transcriptional expression of different ABCCs family members in 20 types of cancer. The data was compared by t-test. The cut-off *P* value and the fold change were as follows: *P* value <.0001, fold change = 1.5, gene grade = 10%. Red means overexpression, blue means overexpression.

3.2. Functional enrichment of ABCCs in LIHC

We constructed a network of ABCCs and their 20 related genes using GeneMANIA (Fig. 8A). ABCC members interacted with the following proteins: ABCB11, ABCB1, ABCB4, ABCB5, ABCD2, ABCD3, ABCD4, ABCB6, ABCB7, ABCB8, ABCB10, ABCB9, ABCD1, TAP2, TAP1, ABCA10, ABCA12, ABCA8, ABCA5, and ABCA3.

We analyzed the GO functions and pathways of ABCCs and their 20 related genes, via the Bioconductor plugin in R. The top 10 functions and pathways were GO:0042626 (ATPase-coupled transmembrane transporter activity), GO:0015399 (primary active transmembrane transporter activity), GO:0022804 (active transmembrane transporter activity), GO:0016887 (ATPase activity), GO:0140359 (ABC-type transporter activity),

Table 1

Transcriptional expression of ABCCs family members between LIHC and normal liver tissue (Oncomine).

	Types of LIHC vs liver	Fold change	*P* value	*t*-test	Ref
ABCC1	Cirrhosis	1.899	3.86E-5	4.923	Wurmbach Liver[25]
	Cirrhosis	1.770	5.02E-12	9.502	Mas Liver[26]
ABCC4	Hepatocellular Carcinoma	2.186	2.45E-10	8.359	Mas Liver[26]
	Cirrhosis	2.324	6.25E-11	9.954	Mas Liver[26]
	Cirrhosis	2.272	3.89E-6	6.409	Wurmbach Liver[25]
	Hepatocellular Carcinoma	2.321	1.13E-5	4.853	Wurmbach Liver[25]
	Hepatocellular Carcinoma	1.605	7.22E-8	5.495	Chen Liver[27]
	Hepatocellular Carcinoma	2.074	3.38E-34	14.058	Roessler Liver 2[28]
ABCC5	Hepatocellular Carcinoma	2.304	5.23E-9	7.701	Wurmbach Liver[25]
ABCC6	Hepatocellular Carcinoma	1.808	6.12E-11	6.841	Chen Liver[27]
	Hepatocellular Carcinoma	−2.256	2.43E-31	−12.883	Roessler Liver 2[28]
ABCC7	Cirrhosis	9.813	2.02E-8	8.400	Wurmbach Liver[25]
	Cirrhosis	3.519	7.59E-27	16.473	Wurmbach Liver[25]
	Hepatocellular Carcinoma	1.800	7.45E-7	5.541	Mas Liver26
	Hepatocellular Carcinoma	−2.019	9.57E-26	−11.217	Roessler Liver 2[28]
ABCC9	Hepatocellular Carcinoma	−5.857	5.48E-13	−9.922	Wurmbach Liver[25]
	Cirrhosis	−7.525	5.11E-13	−15.057	Wurmbach Liver[25]
	Liver Cell Dysplasia	−3.146	5.49E-6	−5.632	Wurmbach Liver[25]
	Hepatocellular Carcinoma	−1.630	1.02E-10	−8.376	Roessler Liver[28]
ABCC10	Hepatocellular Carcinoma	2.553	9.77E-8	7.557	Wurmbach Liver[25]
	Hepatocellular Carcinoma	1.839	4.47E-47	16.858	Roessler Liver 2[28]
	Hepatocellular Carcinoma	1.561	1.16E-5	5.232	Roessler Liver[28]

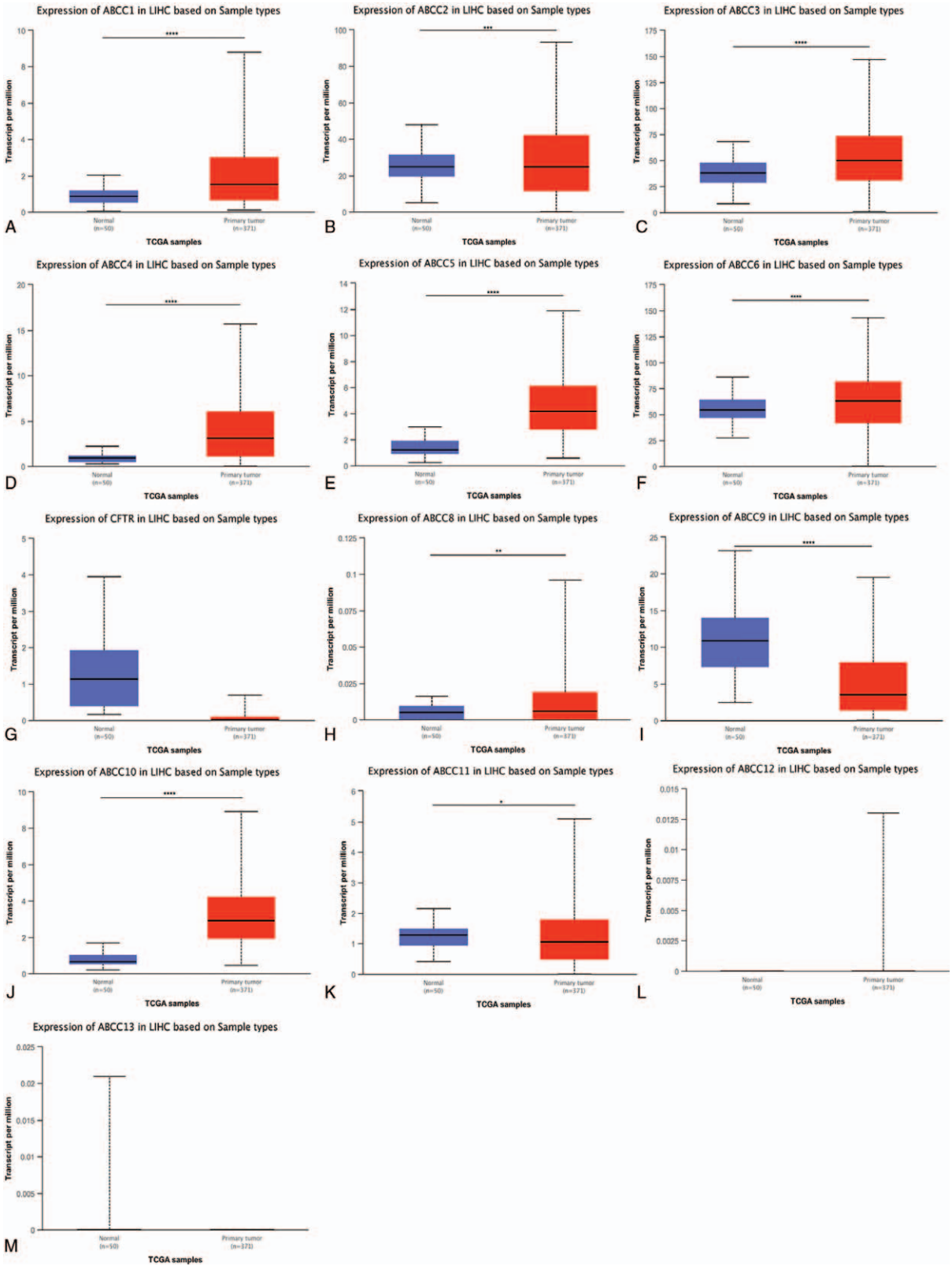

Figure 2. mRNA expression of different ABCCs family members in LIHC patients and normal liver tissues. The mRNA expression of different ABCCs family members in LIHC patients from the TCGA database (A–M). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$.

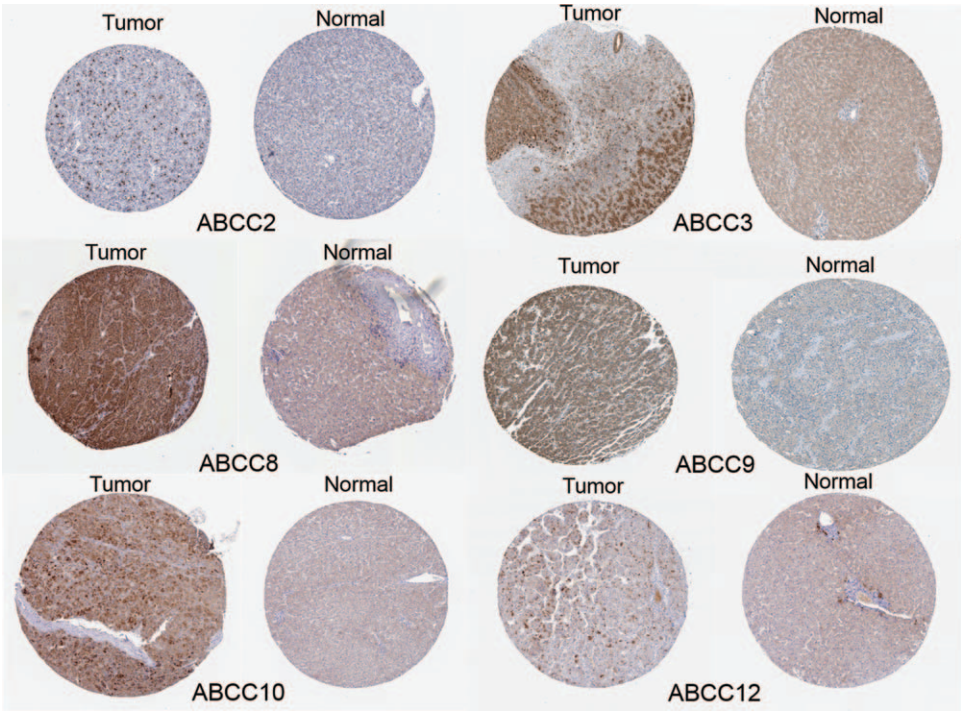

Figure 3. Representative immunohistochemical images of different ABCCs family members in LIHC tissues and normal liver tissues (HPA database). The expression of ABCC3, ABCC4, ABCC8 and ABCC9 increased, and the expression of ABCC2 and ABCC12 decreased.

GO:0042910 (xenobiotic transmembrane transporter activity), GO:0008559 (ABC-type xenobiotic transporter activity), GO:0005319 (lipid transporter activity), GO:0008509 (anion transmembrane transporter activity), and GO:0022853 (active ion transmembrane transporter activity) (Fig. 8B, $P < .05$). The primary enriched KEGG pathways were as follows: ABC transporters, Bile secretion, Antifolate resistance, and Peroxisome (Fig. 8C; $P < .05$).

3.3. Correlation between ABCC mRNA expression and LIHC immune infiltration

We used the TIMER database to determine the correlation between ABCC mRNA expression and the level of immune infiltration in LIHC (Fig. 9). The mRNA expression of members ABCC1/4/5/10 was positively correlated with B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil, and dendritic cell infiltration (Fig. 9A, D, E, J; $P < .05$). ABCC6/7 was negatively correlated with infiltration by these cells (Fig. 9F, G; all $P < .01$). mRNA expression of ABCC2 was negatively correlated with CD8+ T cell infiltration (Fig. 9B; $P < .001$); that of ABCC3 was positively correlated with CD4+ T cell, macrophage, and neutrophil infiltration (Fig. 9C; $P < .001$); that of ABCC8 was positively correlated with CD4+ T cell and macrophage infiltration (Fig. 9H; $P < .001$); that of ABCC9 was negatively correlated with B cell and macrophage infiltration (Fig. 9I; $P < .001$); and that of ABCC11 was negatively correlated with B cell infiltration (Fig. 9K; $P < .05$). There were no correlations between ABCC12/13 mRNA expression and immune-cell infiltration (Fig. 9M, L; $P \geq .05$). In summary, for most of the ABCC members, mRNA expression was correlated with immune-cell infiltration in LIHC.

3.4. Correlation between ABCC mRNA expression and OS

ABCC mRNA expression was associated with OS in LIHC patients. Poor prognosis was associated with high mRNA expression of members ABCC1/4/5/8 (Fig. 10A, D, E, H; $P < .05$) and low mRNA expression of members ABCC6/7/9/12/13 (Fig. 10F, G, I, L, M; $P < .05$).

ABCC2 and ABCC13 showed prognostic significance in both men and women (Table 2; all $P < .05$). ABCC1/5–9/12 showed prognostic significance in men (ABCC7, $P < .05$; the others, $P < .01$), and ABCC2/13 showed prognostic significance in women ($P < .05$).

ABCC1/6/12/13 had prognostic significance for tumor grades 1 to 3 ($P < .05$), ABCC5/7 for grades 2/3 ($P < .05$), and ABCC3/4/8/11 for grade 2 ($P < .05$). Tumor grade 4 was excluded because of its small sample size (n = 12) (Table 3).

We combined tumor stages 3 and 4, because of the small sample size of stage 4 (n = 4). ABCC5/11/12/13 showed prognostic significance for all stages ($P < .05$), ABCC1/8 for stage 1 ($P < .01$), ABCC6/7 for stage 2 ($P < .05$), and ABCC4/6/9/10 for the combined stage 3 + 4 ($P < .05$) (Table 4).

ABCC1/11/12/13 showed prognostic significance for AJCC T stages 1–3 ($P < .05$), ABCC5/ABCC6 for AJCC T 2 and 3 ($P < .01$), ABCC10 for AJCC T 1 ($P < .05$), ABCC7 for AJCC T 2 ($P < .05$), and ABCC4/9/10 for AJCC T 3 ($P < .05$) (Table 5). We excluded AJCC T 4 because of its small sample size (n = 13).

ABCC1/5/6/13 showed prognostic significance for vascular and microvascular invasion ($P < .05$), ABCC4/12 for vascular invasion ($P < .01$), and ABCC3/8/9/11 for microvascular invasion ($P < .05$) (Table 6). We did not analyse macrovascular invasion because of its small sample size (n = 16).

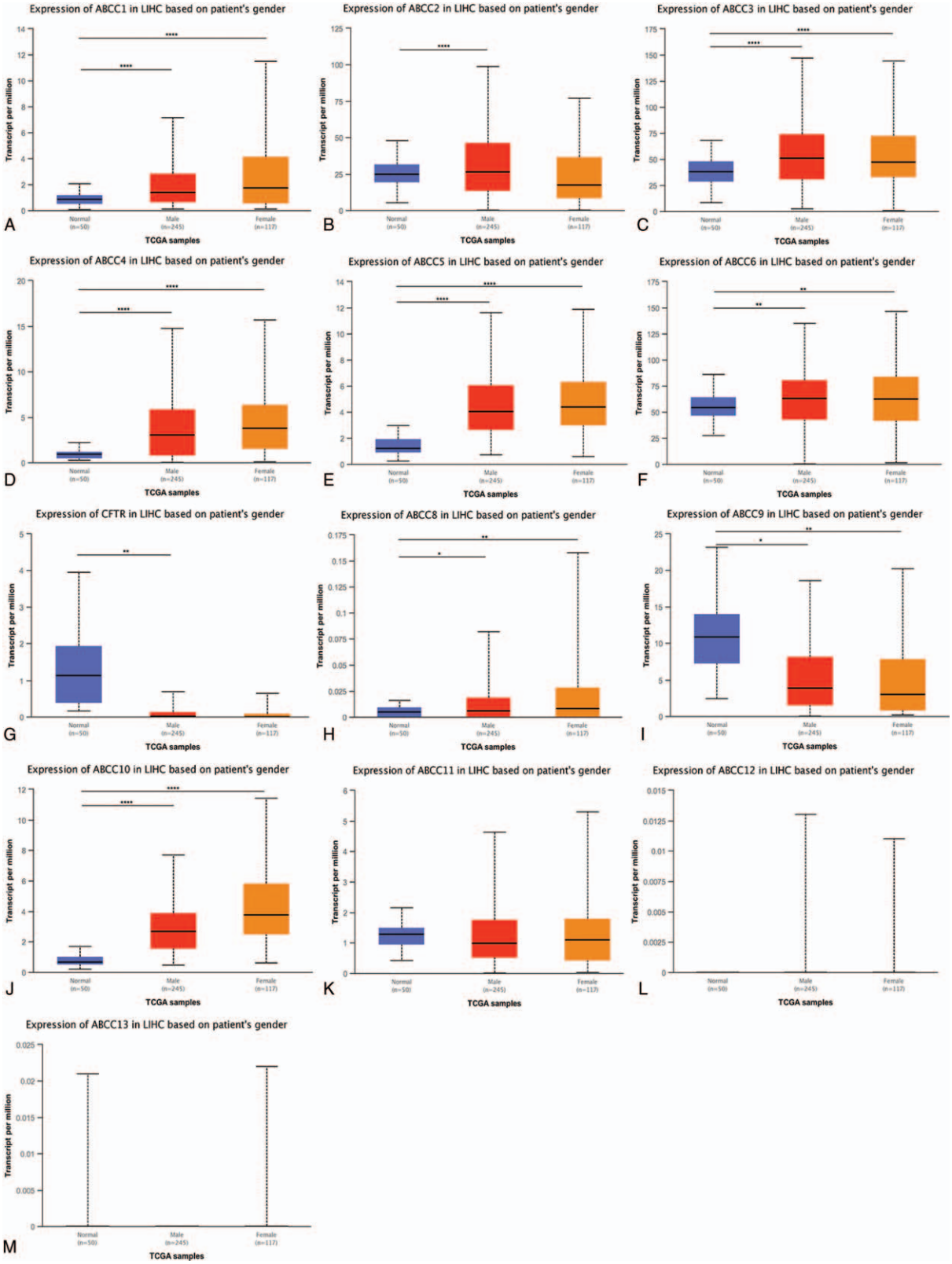

Figure 4. The relationship between the mRNA expression of ABCCs family members and the sex of LIHC patients. Box plots showed the mRNA expression (A–M) of family members of ABCCs in normal individuals and LIHC patients of different genders. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$, $^{****}P < .0001$.

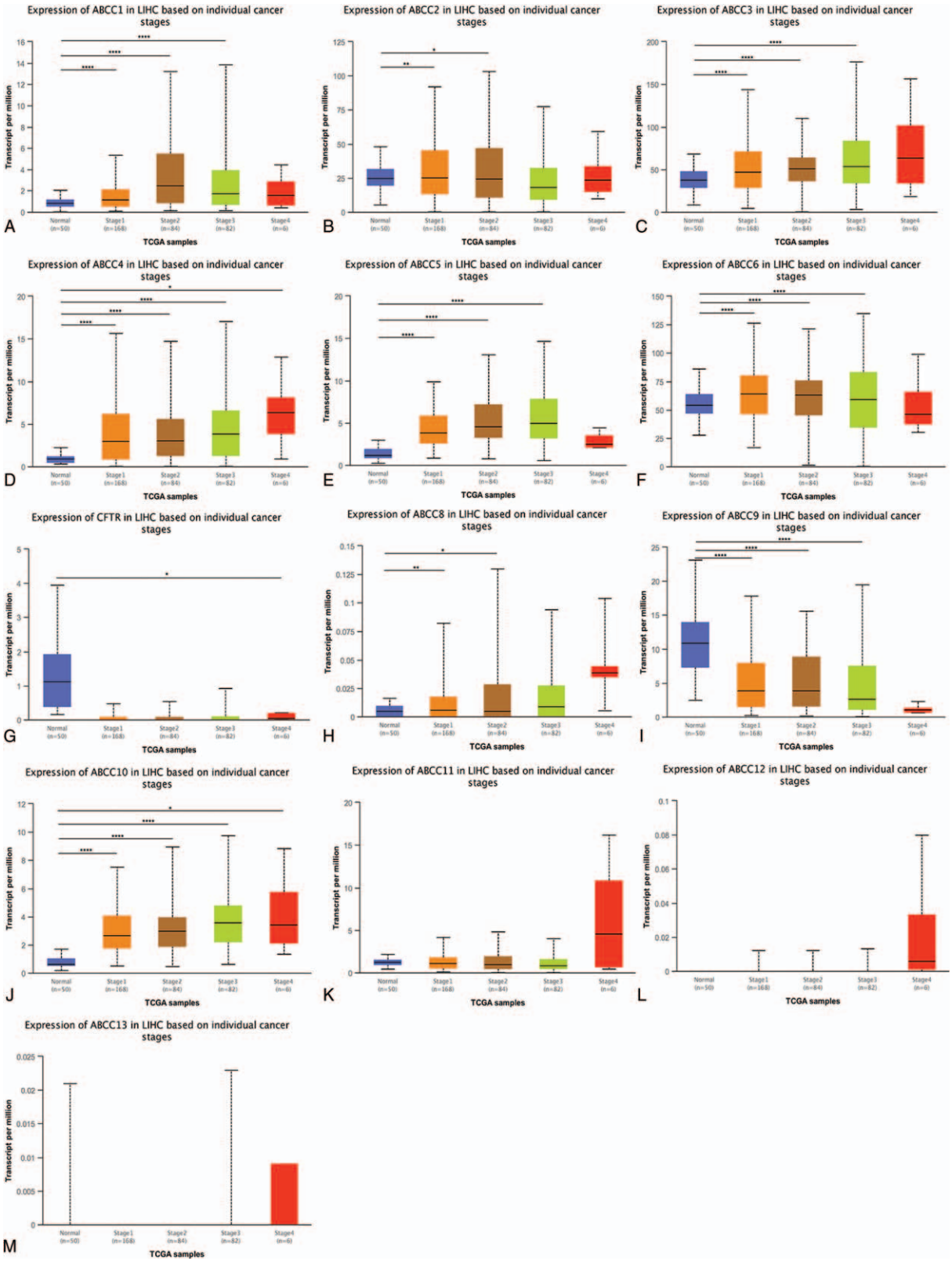

Figure 5. The mRNA expression of ABCCs family members is correlated with the tumor grade of LIHC. The box plot showed the normal individuals or LIHC patients in Grade 1: Well differentiated (low grade), Grade 2: Moderately differentiated (intermediate grade), Grade 3: Poly differentiated (high grade) or Grade 4: Undifferentiated (high grade) (A–M) mRNA expression of ABCCs family members. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$, $^{****}P < .0001$.

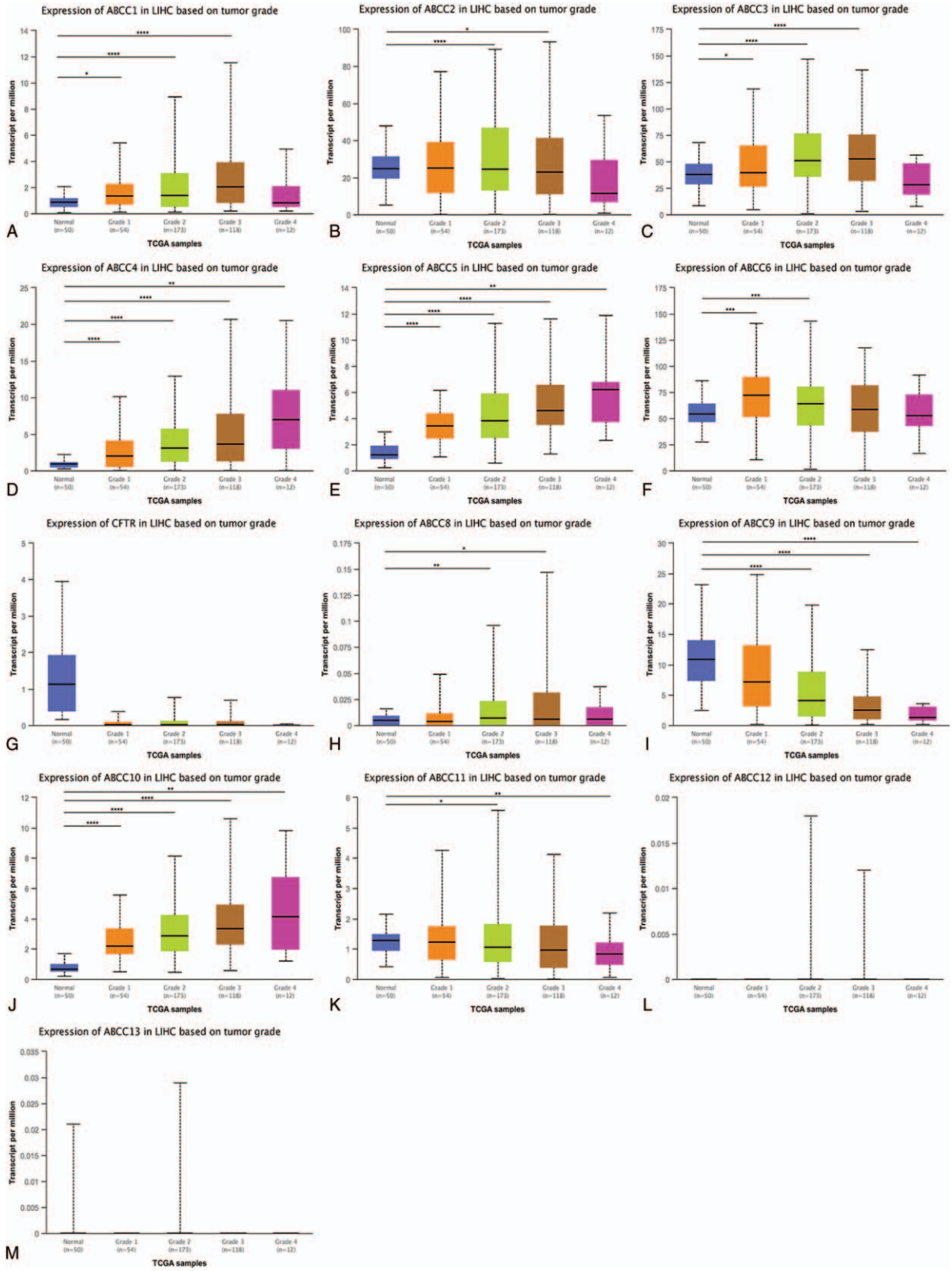

Figure 6. The mRNA expression of ABCCs family members is correlated with the tumor stage of LIHC patients. The box plot shows the mRNA expression of ABCCs family members in normal individuals and LIHC patients in stage 1, stage 2, stage 3 and stage 4 (A–M). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$, $^{****}P < .0001$.

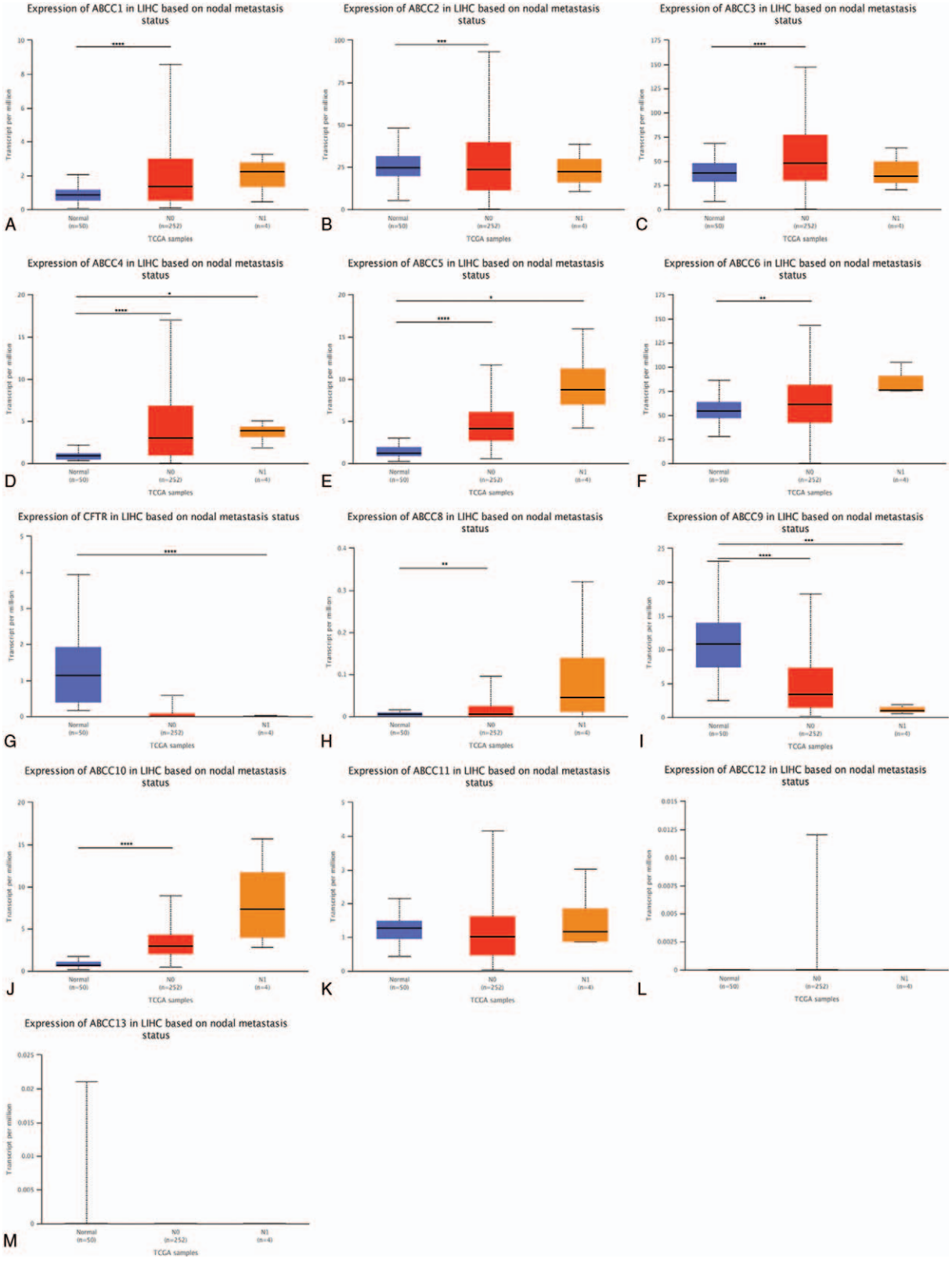

Figure 7. The mRNA expression of ABCCs family members is correlated with the status of lymph node metastasis in patients with LIHC. The box plot showed the mRNA expression of ABCCs family members in normal individuals or lymph node metastasis states N0 or N1 (A–M). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$, $^{****}P < .0001$.

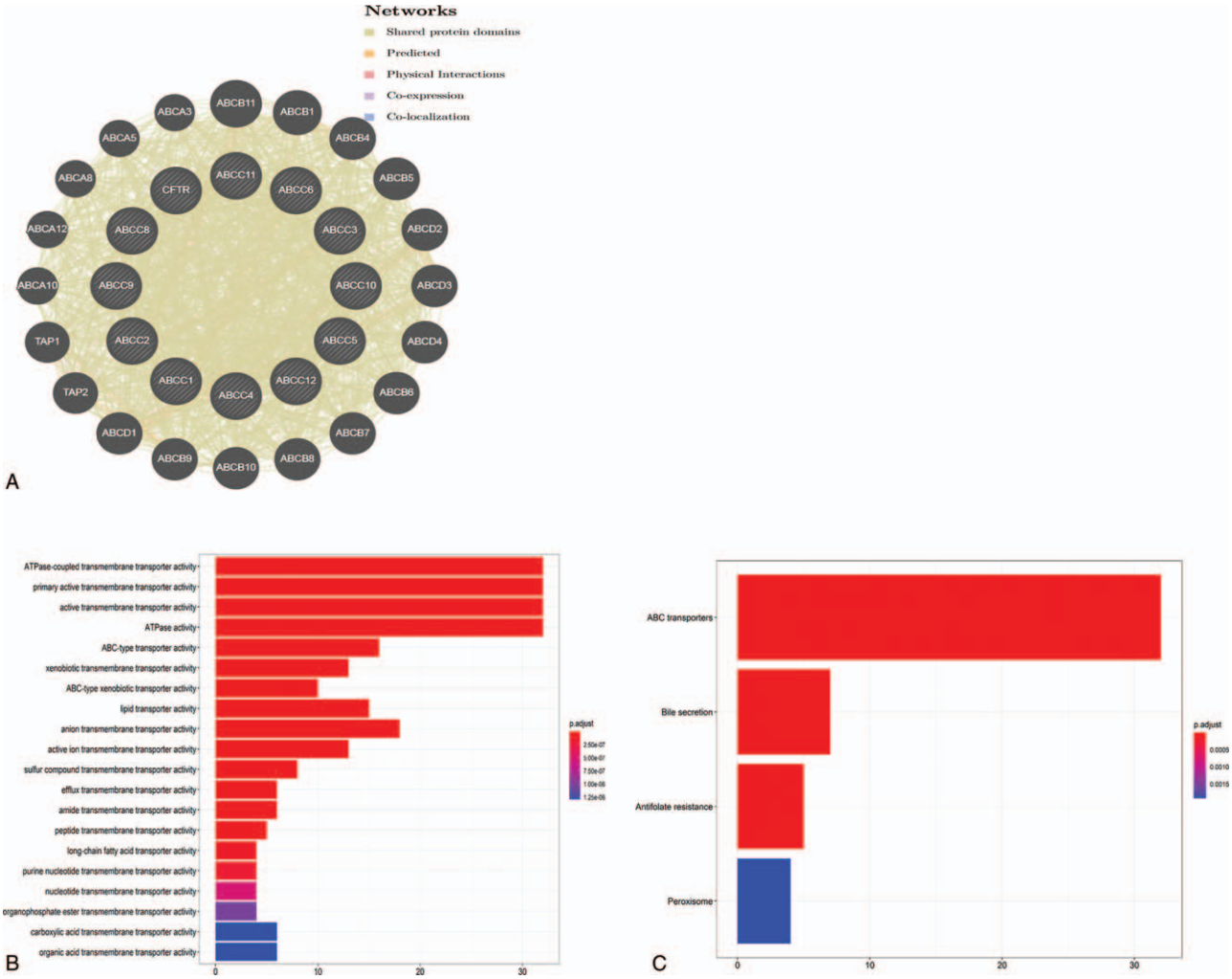

Figure 8. Functional enrichment of members of the ABCCs family in LIHC. A. Analyze the network of ABCCs family members and their 20 related genes by GeneMANIA. B. GO enrichment analysis ranked the top 20 pathways; C. KEGG pathway analysis.

4. Discussion

Novel diagnostic and prognostic markers are urgently required in LIHC, and prior work has suggested ABCCs as promising candidates. The objective of this study was to describe the roles and mechanisms of action of ABCCs in LIHC. We established the diagnostic value of ABCCs in LIHC by comparing their mRNA expression in LIHC and normal (noncancerous) liver tissue: ABCC1/2/3/4/5/6/10 were upregulated, and ABCC9/11 downregulated, in LIHC. ABCC mRNA expression was associated with gender, grade, stage, and lymph node metastasis status. ABCC1–9/10/11 therefore provide potential diagnostic markers for LIHC. We found that ABCCs interact mainly with ABCB11, ABCB1, ABCB4, ABCB5, and other ABCCs, and function by, for instance, participating in ATPase-coupled transmembrane transporter activity and interacting with ABC transporters.

Our findings show that ABCCs are potential targets for LIHC immunotherapy: ABCC mRNA expression was correlated with B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil, and dendritic cell infiltration. This indicates that ABCCs play a key role in LIHC, possibly by regulating the immune response. Further, our findings reveal the prognostic value of ABCCs in LIHC: poor prognosis was associated with high mRNA expression of ABCC1/4/5/8 and low expression of ABCC6/7/9/12/13. Upregulated mRNA expression was observed for ABCC2/13 in both men and women; for ABCC1/6/12/13 in tumor grades 1–3; for ABCC5/11/12/13 in all tumor stages; and for ABCC1/11/12/13 in AJCC T stages 1–3. ABCC1/5/6/13 showed prognostic significance in vascular and microvascular invasion.

Our finding that ABCC expression is disrupted in LIHC, and that this family has prognostic value, is consistent with prior findings. ABCC1 is overexpressed in non-small cell lung cancer tissue, serum, and cells;[53] further, it is significantly highly expressed in breast cancer.[54] ABCC2 is significantly highly expressed in ovarian cancer.[55] ABCC3 is upregulated in the malignant ascites of ovarian cancer, possibly due to the growth of ovarian cancer spheroids.[56] ABCC3 and ABCC6 expression is higher in high-grade than in low-grade serous carcinoma.[57] ABCC4 is overexpressed in colorectal cancer, in which it may be associated with phenotypic transition, which regulates cell migration in a cyclic nucleotide-dependent manner.[58] ABCC5 is significantly overexpressed in prostate cancer, in which its expression is positively correlated with cell proliferation, migration, and invasion.[29]In esophageal squamous cell carcinoma, ABCC7 overexpression can activate the p38 signaling

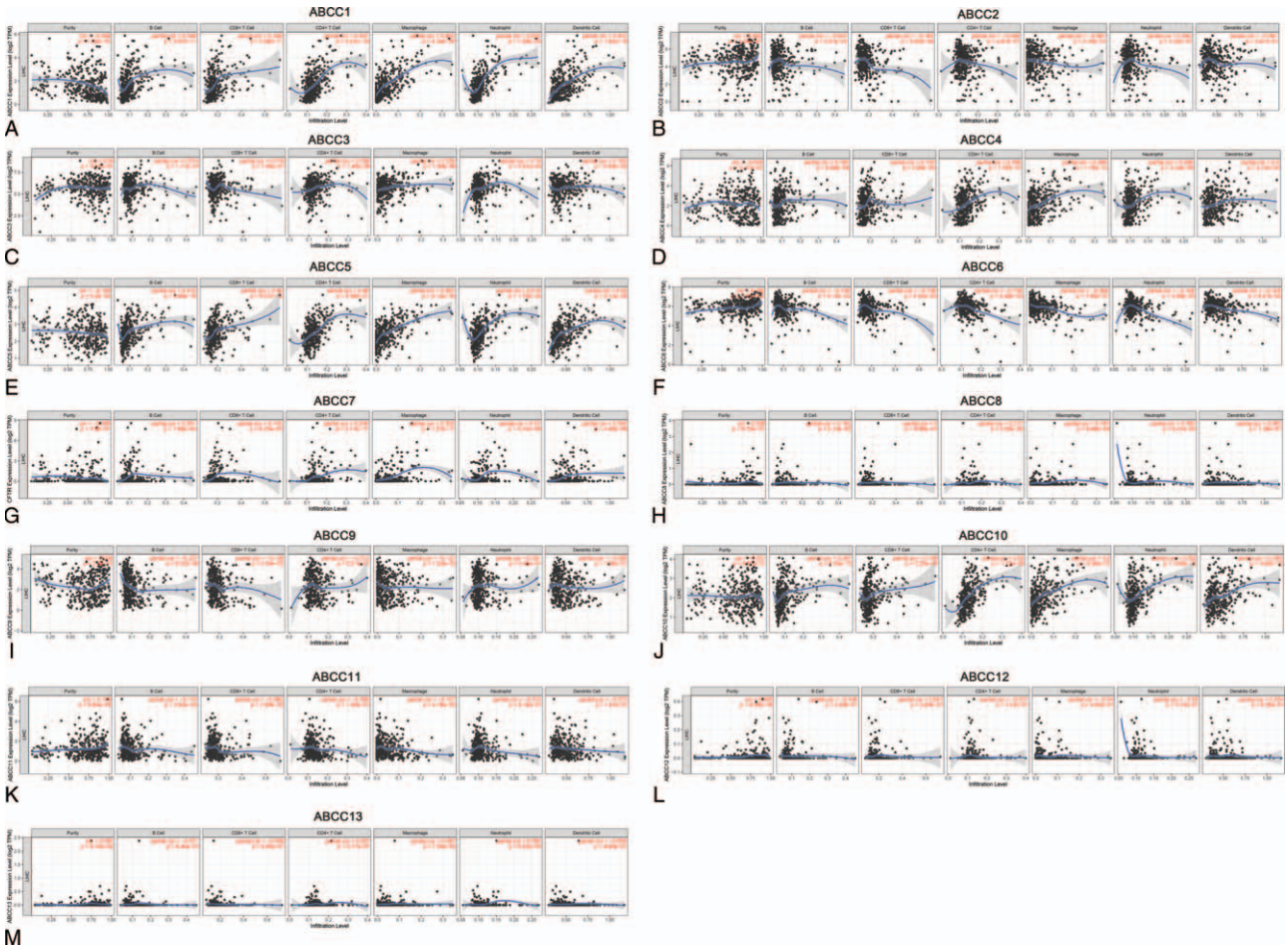

Figure 9. The relationship between the mRNA expression of ABCCs family members and the level of immune infiltration in LIHC. The mRNA expression of ABCCs family members were significantly correlated with the level of immune infiltration in LIHC (A–M).

pathway; this activation is associated with a good prognosis.[59] ABCC8 mRNA expression is a new independent prognostic indicator of glioma: high expression is associated with longer survival.[60] ABCC9 is downregulated in prostate cancer.[61] In colorectal cancer, ABCC10 downregulation reduces survival, and low ABCC11 protein expression increases the risk of cancer recurrence.[62] ABCC12 may become a useful target for breast cancer immunotherapy: although it is not expressed in normal (noncancerous) breast tissue, it is highly expressed in breast cancer.[63] Little is known about ABCC13 expression in relation to tumors; however, it is highly expressed in human fetal liver.[64]

To the best of our knowledge, we are the first to determine that ABCCs can be used as markers for the diagnosis, treatment, and prognosis of LIHC, providing new ideas and targets to this end. Prior work has revealed that ABCC1/2/3 are associated with LIHC diagnosis and prognosis,[65,66] and that ABCCs may be upregulated in untreated LIHC tissue, mediated by cellular microRNAs.[67] However, these studies examined a limited number of ABCC members and associations, without addressing mRNA or protein expression, molecular function, immune infiltration, or prognosis. Because they were based on animal and human experiments, these studies had research biases. Further, their samples were too small to adequately describe the diagnostic and prognostic value of ABCCs.

Our study has different limitations. First, the analysis was database-driven and retrospective. Second, for some ABCC members, there was insufficient transcription and expression data. Our selection of subgroups and of the study sample may have introduced biases into the analysis. Our future experimental and clinical prospective research will address these limitations. To verify these findings, studies using animal experiments and larger cohorts are needed.

5. Conclusion

To the best of our knowledge, this is the first study to identify ABCCs as potential markers for LIHC diagnosis, treatment, and prognosis. We identified ABCC1/2/3/4/5/6/9/10/11 as potential diagnostic markers, and ABCC1/4/5/6/7/8/9/1/13 as prognostic markers for LIHC. Although much remains to be discovered about the roles of ABCCs in LIHC, this work provides insight and potential targets for the diagnosis and treatment of LIHC. Our future work will promote the use of ABCCs in the diagnosis and treatment of LIHC.

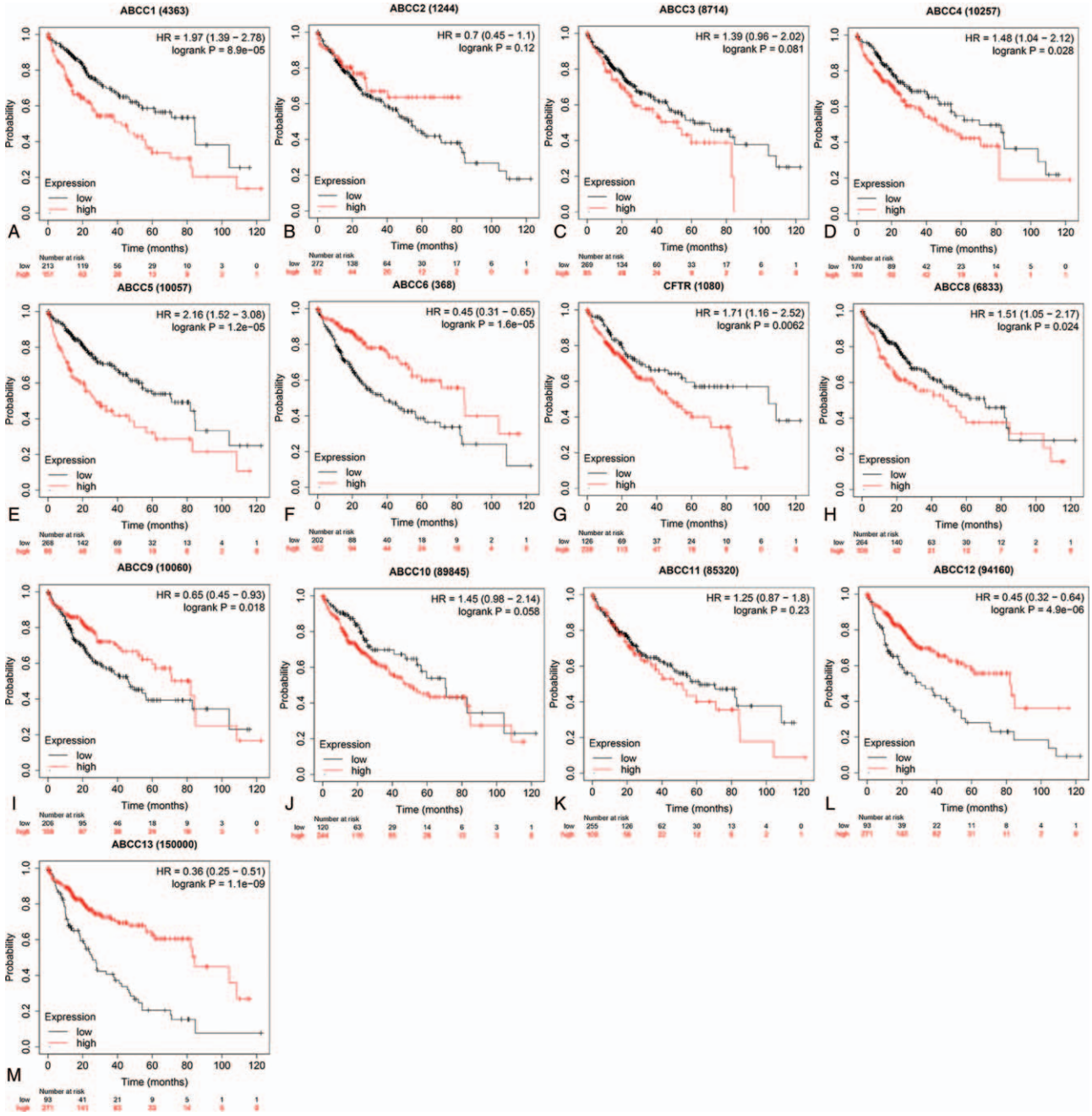

Figure 10. The prognostic value of mRNA expression of ABBCs family members in LIHC patients. Compare the survival curves of high and low expression of ABBCs family members of LIHC patients in Kaplan–Meier plotter.

Table 2

Correlation analysis between ABCCs and gender.

Gene	Gender	Cases	HR	95% CI	*P* value
ABCC1	Male	246	2.71	**1.74–4.24**	**.0000051**
	Female	118	0.71	0.39–1.27	.2449
ABCC2	Male	246	0.57	**0.34–0.96**	**.0337**
	Female	118	1.97	**1.1–3.5**	**.0193**
ABCC3	Male	246	1.37	0.86–2.18	.1857
	Female	118	1.45	0.73–2.86	.2868
ABCC4	Male	246	1.52	0.97–2.38	.0643

(*continued*)

Table 2

(continued).

Gene	Gender	Cases	HR	95% CI	*P* value
	Female	118	1.8	0.98–3.45	.075
ABCC5	Male	246	2.61	**1.67–4.09**	**.000012**
	Female	118	1.86	0.93–3.72	.0757
ABCC6	Male	246	0.35	**0.23–0.55**	**.000002**
	Female	118	0.53	0.27–1.03	.0579
ABCC7	Male	246	1.98	**1.16–3.35**	**.0101**
	Female	118	1.63	0.88–3.02	.1195
ABCC8	Male	246	1.88	**1.18–2.98**	**.0067**
	Female	118	0.75	0.42–1.34	.3235
ABCC9	Male	246	0.42	**0.25–0.72**	**.001**
	Female	118	1.48	0.82–2.67	.1906
ABCC10	Male	246	1.41	0.88–2.26	.1464
	Female	118	1.72	0.91–3.25	.0925
ABCC11	Male	246	1.34	0.85–2.12	.1991
	Female	118	1.72	0.93–3.17	.0819
ABCC12	Male	246	0.31	**0.2–0.48**	**.000000034**
	Female	118	1.57	0.87–2.84	.1294
ABCC13	Male	246	0.27	**0.17–0.42**	**7.1E-10**
	Female	118	0.46	**0.26–0.83**	**.0083**

Table 3

Correlation analysis between ABCCs and staging.

GENE	Grade	Cases	HR	95% CI	*P* value
ABCC1	1	55	0.31	**0.1–0.94**	**.0286**
	2	174	2.59	**1.53–4.38**	**.00025**
	3	118	1.97	**1.01–3.83**	**.0429**
	4	12	–	–	–
ABCC2	1	55	1.65	0.61–4.45	.3199
	2	174	0.56	0.29–1.08	.0804
	3	118	1.34	0.73–2.44	.3449
	4	12	–	–	–
ABCC3	1	55	2.51	0.88–7.11	.0742
	2	174	1.84	**1.07–3.14**	**.0241**
	3	118	0.58	0.31–1.1	.0895
	4	12	–	–	–
ABCC4	1	55	2.36	0.93–5.99	.0629
	2	174	1.79	**1.03–3.11**	**.0352**
	3	118	1.29	0.66–2.52	.4495
	4	12	–	–	–
ABCC5	1	55	1.75	0.66–4.63	.2534
	2	174	2.39	**1.44–3.97**	**.00055**
	3	118	2.76	**1.51–5.06**	**.00058**
	4	12	–	–	–
ABCC6	1	55	0.18	**0.07–0.47**	**8.90E-05**
	2	174	0.56	**0.33–0.95**	**.0286**
	3	118	0.32	**0.14–0.72**	**.0039**
	4	12	–	–	–
ABCC7	1	55	2.14	0.84–5.48	.105
	2	174	2.24	**1.2–4.18**	**.009**
	3	118	0.45	**0.21–0.93**	**.0273**
	4	12	–	–	–
ABCC8	1	55	0.51	0.19–1.35	.1696
	2	174	1.81	**1.08–3.04**	**.0233**
	3	118	1.65	0.85–3.18	.1333
	4	12	–	–	–
ABCC9	1	55	0.37	0.13–1.08	.0578
	2	174	0.65	0.39–1.1	.1069
	3	118	0.51	0.23–1.15	.1001
	4	12	–	–	–

(*continued*)

Table 3

(continued).

GENE	Grade	Cases	HR	95% CI	*P* value
ABCC10	1	55	2.57	0.97–6.83	.0505
	2	174	1.49	0.89–2.49	.1248
	3	118	1.38	0.74–2.57	.3051
	4	12	–	–	–
ABCC11	1	55	0.46	0.18–1.21	.1057
	2	174	1.98	**1.18–3.33**	**.0086**
	3	118	0.65	0.35–1.21	.1711
	4	12	–	–	–
ABCC12	1	55	0.39	**0.15–1**	**.0436**
	2	174	0.43	**0.25–0.72**	**.0011**
	3	118	0.46	**0.25–0.84**	**.0102**
	4	12	–	–	–
ABCC13	1	55	0.27	**0.1–0.69**	**.0033**
	2	174	0.31	**0.19–0.52**	**3.00E-06**
	3	118	0.33	**0.18–0.61**	**.00017**
	4	12	–	–	–

Table 4

Correlation analysis between ABCCs and tumor grade.

GENE	Stage	Cases	HR	95% CI	*P* value
ABCC1	1	170	2.4	**1.29–4.47**	**.0045**
	2	83	2.23	0.96–5.17	.0559
	3+4	87	1.71	0.91–3.21	.0899
ABCC2	1	170	1.84	0.98–3.47	.0552
	2	83	1.81	0.81–4.07	.1443
	3+4	87	0.67	0.35–1.28	.2236
ABCC3	1	170	1.49	0.78–2.83	.2261
	2	83	0.6	0.27–1.33	.2079
	3+4	87	0.57	0.32–1.04	.0634
ABCC4	1	170	1.44	0.77–2.71	.2489
	2	83	1.94	0.86–4.35	.1019
	3+4	87	2.07	**1.02–4.19**	**.0396**
ABCC5	1	170	3.35	**1.41–7.95**	**.0036**
	2	83	4.03	**1.83–8.85**	**.00018**
	3+4	87	2.36	**1.3–4.3**	**.0039**
ABCC6	1	170	0.59	0.32–1.08	.0831
	2	83	0.31	**0.12–0.78**	**.0086**
	3+4	87	0.39	**0.21–0.75**	**.0036**
ABCC7	1	170	1.66	0.87–3.16	.1174
	2	83	2.84	**1.11–7.3**	**.0242**
	3+4	87	0.63	0.33–1.22	.1682
ABCC8	1	170	3.32	**1.39–7.95**	**.0043**
	2	83	0.59	0.26–1.35	.2094
	3+4	87	0.62	0.32–1.19	.1485
ABCC9	1	170	0.78	0.42–1.43	.4186
	2	83	2.09	0.71–6.1	.1693
	3+4	87	0.34	**0.16–0.71**	**.0027**
ABCC10	1	170	2.19	0.97–4.93	.0525
	2	83	0.67	0.3–1.5	.3293
	3+4	87	2.17	**1.1–4.27**	**.0219**
ABCC11	1	170	2.09	**1–4.39**	**.0458**
	2	83	2.25	**1.02–4.97**	**.0393**
	3+4	87	0.5	**0.27–0.92**	**.0233**
ABCC12	1	170	0.51	**0.27–0.95**	**.0312**
	2	83	0.37	**0.17–0.81**	**.0101**
	3+4	87	0.43	**0.24–0.78**	**.0041**
ABCC13	1	170	0.41	**0.22–0.75**	**.0029**
	2	83	0.37	**0.17–0.81**	**.0092**
	3+4	87	0.4	**0.22–0.72**	**.0018**

Table 5

Correlation analysis between ABCCs and AJCC T classification.

GENE	AJCC_T	Cases	HR	95% CI	*P*-value
ABCC1	1	180	2.1	**1.15–3.82**	**.0129**
	2	90	2.52	**1.12–5.7**	**.0214**
	3	78	1.97	**1.04–3.73**	**.034**
	4	13	–	–	–
ABCC2	1	180	1.71	0.94–3.11	.078
	2	90	2.02	0.96–4.29	.0609
	3	78	0.66	0.35–1.25	.2009
	4	13	–	–	–
ABCC3	1	180	1.37	0.73–2.59	.32
	2	90	1.7	0.64–4.5	.2817
	3	78	0.6	0.32–1.12	.1048
	4	13	–	–	–
ABCC4	1	180	1.46	0.8–2.67	.2154
	2	90	2.05	0.97–4.31	.0543
	3	78	2.04	**1–4.18**	**.0461**
	4	13	–	–	–
ABCC5	1	180	2.42	1.17–5.02	.0143
	2	90	3.62	**1.75–7.47**	**.0002**
	3	78	2.89	**1.53–5.43**	**.00063**
	4	13	–	–	–
ABCC6	1	180	0.61	0.34–1.09	.0933
	2	90	0.28	**0.11–0.69**	**.0031**
	3	78	0.39	**0.2–0.77**	**.0048**
	4	13	–	–	–
ABCC7	1	180	1.74	0.86–3.53	.1216
	2	90	3.02	**1.2–7.63**	**.0144**
	3	78	1.64	0.84–3.22	.15
	4	13	–	–	–
ABCC8	1	180	3.17	1.41–7.15	3.17
	2	90	0.61	0.28–1.31	.1989
	3	78	1.72	0.92–3.21	.0877
	4	13	–	–	–
ABCC9	1	180	0.71	0.4–1.27	.2509
	2	90	1.8	0.73–4.42	.1948
	3	78	0.31	**0.14–0.68**	**.0021**
	4	13	–	–	–
ABCC10	1	180	2.2	**0.98–4.91**	**.0496**
	2	90	1.29	0.61–2.73	.5053
	3	78	2.94	**1.37–6.31**	**.0041**
	4	13	–	–	–
ABCC11	1	180	2.28	**1.09–4.74**	**.0237**
	2	90	2.18	**1.04–4.57**	**.0347**
	3	78	0.52	**0.27–0.99**	**.0432**
	4	13	–	–	–
ABCC12	1	180	0.5	**0.27–0.91**	**.0202**
	2	90	0.44	**0.21–0.91**	**.024**
	3	78	0.46	**0.25–0.84**	**.0104**
	4	13	–	–	–
ABCC13	1	180	0.43	**0.24–0.77**	**.00238**
	2	90	0.37	**0.18–0.76**	**.0048**
	3	78	0.37	**0.19–0.7**	**.0016**
	4	13	–	–	–

Table 6

Correlation analysis between ABCCs and Vascular invasion.

GENE	Vascular invasion	Cases	HR	95% CI	*P* value
ABCC1	None	203	1.92	**1.13–3.28**	**.0149**
	Micro	90	2.24	**1.02–4.9**	**.038**
	Macro	16	–	–	–
ABCC2	None	203	1.42	0.83–2.42	.198
	Micro	90	0.56	0.25–1.23	.1397

(*continued*)

Table 6
(continued).

GENE	Vascular invasion	Cases	HR	95% CI	*P* value
	Macro	16	–	–	–
ABCC3	None	203	1.39	0.8–2.39	.2395
	Micro	90	2.35	**1.08–5.09**	**.0261**
	Macro	16	–	–	–
ABCC4	None	203	1.79	**1.04–3.08**	**.035**
	Micro	90	1.37	0.62–3.04	.4307
	Macro	16	–	–	–
ABCC5	None	203	1.72	**1.03–2.87**	**.0366**
	Micro	90	2.92	**1.35–6.31**	**.0043**
	Macro	16	–	–	–
ABCC6	None	203	0.48	**0.28–0.83**	**.0067**
	Micro	90	0.32	**0.11–0.93**	**.027**
	Macro	16	–	–	–
ABCC7	None	203	1.64	0.89–3.02	.1073
	Micro	90	0.41	0.14–1.18	.088
	Macro	16	–	–	–
ABCC8	None	203	1.35	0.78–2.34	.2849
	Micro	90	3.01	**1.4–6.45**	**.003**
	Macro	16	–	–	–
ABCC9	None	203	0.77	0.45–1.31	.3293
	Micro	90	0.43	**0.18–1.01**	**.0451**
	Macro	16	–	–	–
ABCC10	None	203	1.77	0.97–3.23	.0599
	Micro	90	0.52	0.24–1.11	.0868
	Macro	16	–	–	–
ABCC11	None	203	1.65	0.91–2.97	.0932
	Micro	90	2.29	**1.05–5**	**.0319**
	Macro	16	–	–	–
ABCC12	None	203	0.35	**0.21–0.6**	**4.60E-05**
	Micro	90	1.85	0.86–4	.1116
	Macro	16	–	–	–
ABCC13	None	203	0.33	**0.19–0.54**	**7.00E-06**
	Micro	90	0.35	**0.16–0.74**	**.0044**
	Macro	16	–	–	–

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