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H．知的財産権の出願・登録状況（予定を含む。）
1．特許取得
なし
2．実用新案登録
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3．その他
なし

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書
胃癌の腹膜播種に対する標準的治療の確立

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研究要旨　胃癌の腹膜播種は、様々合併症を有する患者が多く、臨床試験を行うのが難しいとされ、その標準的治療は確立していない。本研究班では、胃癌腹膜播種例に対する標準的治療の確立を目指した 5FU 持続静注(5Fuci)vsMTX+5FU 時間差療法(MF)の第Ⅲ相試験 (JCOG0106)や二次治療の開発を目指した best available 5FU vs Paclitaxel 少量分割療法のランダム化第Ⅱ相試験が行われ、いずれも登録終了し結果解析中である。当院も分担研究者として、両試験に参加し協力してきた。本研究結果により、胃癌腹膜播種患者に対する標準的治療確立及び予後の向上が期待される。

A. 研究目的

胃癌腹膜播種に対する標準的治療の確立を目指してMTX+5FU時間差療法の有用性を検討するため 5FU 単独持続療法との比較すること。2 次治療としての Paclitaxel 少量分割療法の有効性と安全性の評価。

B. 研究方法

JCOG0106 (MTX+5FU vs 5Fuci, phase Ⅲ)、JCOG0407 (best available 5FU vs weekly paclitaxel)、いずれの試験も primary endpoint は全生存期間。JCOG0106 は、初回治療例を対象とし、JCOG0407はフッ化ピリミジン系抗がん剤を含んだ初回治療に対して不応になった症例が対象とされた。目標症例数は、JCOG0106 が 236 例、0407 が 100 例。

（倫理面への配慮）

両試験のプロトコールを国立がんセンターの倫理審査委員会に提出し承認が得られた後に研究への登録を開始した。試験の説明は患者本人に行い、文書による同意が得られた後に登録し、試験治療を開始した。

C. 研究結果

いずれの試験も、登録が終了し、最終結果を解析中である。当院からも JCOG0106 に 24 例、0407 に 3 例を登録した。いずれの試験登録例も大きな合併症なく試験治療が遂行できた。

D. 考察

様々な合併症を有し、臨床試験が困難とされてきた胃癌の腹膜播種症例を対象に 2 つの大きな多施設臨床試験を完遂出来たことは非常に意義が大きい。今後、両試験の結果をベースとして、次の試験も構築し治療開発を進めていくことが期待される。

E. 結論

本研究班における 2 つの大きな多施設試験の登録が完遂した。当院からも両試験へ登録し協力することができた。本研究結果により、胃癌腹膜播種患者に対する標準的治療確立及び予後の向上が期待される。

F. 健康危険情報

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G. 研究発表

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1．特許取得
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2．実用新案登録
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分担研究報告書
胃癌の腹膜播種に対する標準的治療の確立

研究分担者　山田　康秀　国立がんセンター中央病院　消化器内科医長

研究要旨　胃癌化学療法の効果予測因子,予後因子としてのインスリン様成長因子-1受容体(IGF-1R)、上皮成長因子受容体(EGFR)、HER2の意義について胃癌原発巣組織を用いて検討し、胃癌治療薬としての抗IGF-1R療法開発の意義に関する知見を得た。

A. 研究目的

進行・再発胃癌の化学療法では、2次治療以降になると癌の増悪による PS の低下に伴い，臨床的に治療可能な症例は大腸癌などに比べると激減する。効率的な治療選択を目標として、腫瘍組織中の細胞増殖因子受容体タンパク発現量と化学療法（S-1単剤、シスプラチン/イリノテカン併用療法、5-FU 単剤など）の効果との相関を解析し，その臨床的意義について検討する。

B. 研究方法

当院で進行胃癌に対し胃切除術が施行されたが遺残またはその後再発し、1995～2004年に化学療法を施行、かつその抗腫瘍効果が判明している全 87 症例を対象とする。ホルマリン固定パラフィン包埋手術標本から作製したプレパラートを用いて免疫組織化学を行い、染色細胞陽性率と各化学療法の抗腫瘍効果との相関を探索解析する。

（倫理面への配慮）

本研究は、タンパク発現量を免疫組織化学により解析するものである。本研究では試料等の提供者に危険・不利益が及ぶ可能性はまずない。その理由は、(1)試料等を厳重に匿名化して行うので、プライバシーの侵害を生じる恐れがないこと、(2)本研究は癌組織における抗癌剤感受性に関する探索的研究であり、一人一人のタンパク発現についての解析により得られる情報だけでは精度や確実性の点で十分に意味がある結果は得られず、あくまでも多数の人々の結果全体を合わせて比較することによりはじめて意味が見えてくることである。さらに本研究は新たな癌治療法の選択を可能にし、同時にそのための簡便な診断法としての評価も行う、重要かつ必要な研究であると受託研究審査委員会が判断している。

C. 研究結果

IGF-1R は 67/87 例(77%)で陽性、EGFR は 55/87 例(63%)、HER2 は 16/87(18%)でそれぞれ陽性であった。IFG-1R と EGFR が共発現している症例は 48/87 例(55%)、IGF-1R と HER2 は 16/87 例(18%)、EGFR と HER2 の共発現は 13/87 例(15%)にみられた。IGF-1R は intestinal type(35/40, 88%)で diffuse type(32/47, 68%)に比べ陽性率が高かった(P=0.002)。これら細胞増殖因子受容体と化学療法の効果に相関はみられなかった。生存期間中央値は、IGF-1R 陽性患者（67/87）で 13.2 ケ月、陰性患者(20/87)で 17.9 ヶ月(P=0.19)、EGFR 陽性(55/87)で 13.5 ヶ月、陰性で 14.7 ヶ月(P=0.97)、HER2 陽性（16/87）で 16.0 ヶ月、陰性で 13.6 ヶ月(P=0.31)であった。多変量解析では、IGF-1R 陽性（ハザード比 2.14、P=0.01)、PS1 または 2（ハザード比

1.83、P=0.01)、組織型 diffuse type（ハザード比 1.71、P=0.02）が有意な予後因子であった。

（d）D. 考察

EGFR は消化器癌（食道癌、胃癌、大腸癌）におけるこれまでの報告では 19%～72%の症例において発現が認められており、ステージ、深達度が進むとともに過剰発現する症例が増加し、患者の予後不良因子であると報告されている。EGFR を標的とするモノクローナル抗体に、セツキシマブ、パニツムマブがあり、現在、大腸がんに対する有効性、延命効果が証明され、胃がんに対する有用性を確認するための臨床試験が行われている。また HER2 陽性乳癌に対して有効なモノクローナル抗体であるトラスツズマブも HER2 陽性胃癌に対する臨床試験が行われている。IGF-1R は胃癌の77%と多くの症例で陽性であり、また陽性患者の予後は不良であるため、EGFR や HER2 同様に抗体療法やチロシンキナーゼ阻害剤による治療法開発の意義があると考えられる。

E. 結論

IGF-1R 陽性胃癌に対する抗 IGF-1R 療法の臨床開発と、IGF-1R 陽性胃癌の特徴を明らかにするために臨床病理学的検討が必要と考えられる。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む。）

なし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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SNP Communication

Genetic Variations and Haplotypes of *ABCC2* Encoding MRP2 in a Japanese Population

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: The multidrug resistance-associated protein 2 (MRP2) encoded by the *ABCC2* gene is expressed in the liver, intestine and kidneys and preferentially exports organic anions or conjugates with glucuronide or glutathione. In this study, all 32 exons and the 5′-flanking region of *ABCC2* in 236 Japanese were resequenced, and 61 genetic variations including 5 novel nonsynonymous ones were detected. A total of 64 haplotypes were determined/inferred and classified into five *1 haplotype groups (*1A, *1B, *1C, *1G, and *1H) without nonsynonymous substitutions and *2 to *9 groups with nonsynonymous variations. Frequencies of the major 4 haplotype groups *1A (−1774delG), *1B (no common SNP), *1C (−24C>T and 3972C>T), and *2 [1249G>A (Val417Ile)] were 0.331, 0.292, 0.172, and 0.093, respectively. This study revealed that haplotype *1A, which has lowered activity, is quite common in Japanese, and that the frequency of *1C, another functional haplotype, was comparable to frequencies in Asians and Caucasians. In contrast, the haplotypes harboring 3972C>T but not −24C>T (*1G group), which are reportedly common in Caucasians, were minor in Japanese. Moreover, the allele 1446C>T (Thr482Thr), which has increased activity, was not detected in our Japanese population. These findings imply possible differences in MRP2-mediated drug responses between Asians and Caucasians.

Keywords: ABCC2; MRP2; genetic variation; haplotype; amino acid change

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As of October 7, 2007, the novel variations reported here are not found in the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).
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Introduction

The multidrug resistance-associated protein 2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT) is a 190–200 kDa transmembrane glycoprotein comprised of 1545 amino acids and belongs to the superfamily C of ATP-binding cassette (ABC) transporters. This transporter is expressed on hepatic canalicular membranes, intestinal apical membranes, luminal membranes of renal proximal tubules, placental epithelial cells, and the blood brain barrier.[1] MRP2 exports endogenous and exogenous substances, preferentially organic anions or conjugates with glucuronide, glutathione and sulfate.[1-3] This protein originally identified in cisplatin-resistant tumor cells[4] is shown to confer drug resistance to other anti-cancer drugs, such as vincristine and doxorubicin.[5,6]

MRP2 is encoded by the *ABCC2* gene located on chromosome 10q24 and consists of 32 exons (31 coding exons) and spans 69 kb. Several *ABCC2* genetic variations have been detected in patients with Dubin-Johnson syndrome (DJS), an autosomal recessive disease characterized by hyperbilirubinemia with conjugated bilirubin or increased coproporphyrin excretion in urine.[2,7] Recent studies on *ABCC2* have identified common single nucleotide polymorphisms (SNPs) such as −24C>T and −3972C>T (Ile 1324Ile) among several ethnic populations, and several studies have suggested their association with altered MRP2 expression or function.[8-17] In more recent studies on *ABCC2* haplotypes covering an extended 5′-flanking region, close linkages were found among −1549A>G in the 5′-flanking region and two common SNPs −24C>T and −3972C>T (Ile1324Ile).[8] In addition, as possible functional SNPs, −1774delG in Koreans[8] and −1019A>G in Caucasians[10] were reported. However, there is little information on detailed haplotype structures throughout the gene, and comprehensive haplotype analysis in Japanese has not yet been conducted.

We previously analyzed *ABCC2* genetic variations within all 32 exons and the proximal 5′-flanking region (approximately 800 bp upstream of the translation initiation site) using established cell lines derived from Japanese cancer patients to obtain preliminary information on *ABCC2* SNPs in Japanese.[18] In this study, to reveal *ABCC2* haplotype structures in Japanese, we resequenced the *ABCC2* gene including the distal 5′-upstream region (approximately 1.9 kb upstream from the translation initiation site) as well as all 32 exons in 236 Japanese subjects and conducted haplotype analysis using the detected genetic polymorphisms.

Materials and Methods

Human DNA samples: Genomic DNA samples were obtained from blood leukocytes of 177 Japanese cancer patients at two National Cancer Center Hospitals (Tokyo and Chiba, Japan) and Epstein-Barr virus-transformed lymphoblastoid cells prepared from 59 healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP consortium (Tokyo, Japan). Written informed consent was obtained from all subjects. Ethical review boards of all participating organizations approved this study.

PCR conditions for DNA sequencing: We sequenced all 32 exons of the *ABCC2* gene and approximately 800 bp upstream of the translation initiation codon (proximal 5′-flanking region) as described previously and also extended the sequenced region to 1.9 kb upstream of the translation initiation site (distal 5′-flanking region). Briefly, for amplification of the proximal 5′-flanking region and 32 exons, 5 sets of multiplex PCR were performed from 200 ng of genomic DNA using 1.25 units of Z-taq (Takara Bio. Inc., Shiga, Japan) with 0.3 uM each of the mixed primers as shown in **Table 1** [1st PCR]. The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72 °C for 190 sec. Next, each exon was amplified separately using the 1st PCR product by Ex-Taq (0.625 units, Takara Bio. Inc.) with appropriate primers (0.3 uM) (**Table 1**) [2nd PCR]. The conditions for the second round PCR were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of the distal 5′-flanking region, multiplex PCR was performed from 25 ng of genomic DNA using 1 unit of Ex-Taq (Takara Bio. Inc.) with 0.4 uM each of the 2 sets of primers as shown in **Table 1** [PCR]. The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min.

Following the PCR, products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx-96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. Genbank NT_030059.12 was used as the reference sequence.

Linkage disequilibrium (LD) and haplotype analyses: Hardy-Weinberg equilibrium and LD analyses were performed using SNPAlyze 3.1 software (Dynacom Co., Yokohama, Japan). Pairwise LDs were shown as rho square (r^2) and |D′| values in **Figure 1**. Diplotype configurations (haplotype combinations) were inferred by LDSUPPORT software, which determined the posterior probability distribution of diplotype configurations for each subject based on estimated haplotype frequencies[19].

Results and Discussion

In this study, sixty-one *ABCC2* genetic variations including 36 novel ones were detected in 236 Japanese subjects

Table 1. Primer sequences used in this study

Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]
PCR (Ex-taq)			
5′-Flanking (for −1.9 k to −1.7 k)	CCACCAGTGCCAAGAGAAGTAT	CACAAGTCATCTGGAAAACACA	20289134–20289443
5′-Flanking (for −1.7 k to −950)	ATGAGGTGGTATCTAACTGTGG	AAATGTTTTCTGTAGGGACGGG	20289392–20290182
1st PCR (Z-taq)			
5′-Flanking (for −1.2 k) to exon 6	ATACTGCATGGGTGGTTATG	AACCTGCCTCCAAATTTTTC	20289942–20303347
Exons 7 to 11	GGAGAATCACTTTGAAGCCG	CTAGCAAGTGTGAGGGGTGT	20304874–20314079
Exons 12 to 19	TCTGTGAATGTGGCAAAACT	GGATCTACCAAGAATTTAGC	20315189–20328004
Exons 20 to 25	GATGAGCATTTTCAATTTAC	TCAGTTCACCCAGCACTTAT	20338211–20344941
Exons 26 to 32	GAGCAAGACCTTGTCTCATA	CCATGGATGAATCTCAGATA	20349821–20360334
2nd PCR (Ex-taq)			
5′-Flanking (for −880 to −130)	GGAAGATCGCTTGAACCCAT	TCATCCCAACCATTTAATCG	20290245–20290994
Exon 1	TTGTTGGCCAGCTCTGTTG	TTCTGGTTCTTGTTGGTGAC	20290810–20291254
Exon 2	GGGTAAGGCTGGATATGGAT	CTGGCTCTACCTGAGACAAT	20292767–20293194
Exon 3	CACCGGAAACCATTCTGTTC	TTTGCCTCACTATGGATCCC	20300442–20300773
Exon 4	GCCAGATTAGTCACGACAGT	CCAAAGGAAGTCTACATGGCC	20301708–20302134
Exon 5	CAGGTAAGGAAAAAAAGAGTGG	CCTTGTCATAAAATGGTCTG	20301966–20302418
Exon 6	TATGCCAGAAAATCTGATTA	AGGTGGAACATGAGCTTGAGT	20302499–20303070
Exon 7	GGTGGAGATAGCCTCTGACC	TGCACTGAGAAGTATGAAGTGC	20305320–20305728
Exon 8	CCTGTACAGAGAAGGCCACG	TGCGGTCTTCATGAACACAA	20307385–20307816
Exon 9	GGCTTTGGACAATTCTGGTC	TCCACCCATTGTCTGTGAAC	20308539–20309038
Exon 10	AGGCAAGAAGTCACAGTGCC	TTGCCCAAACTCCCATTAAG	20312158–20312650
Exon 11	ACAGTCAGGCAAGGGCTATG	GACAGGAGGACATGAAACAA	20313420–20313873
Exon 12	GATTTCTATTCCCCACATTT	GAGCTGGGGGTATGGTACAA	20315554–20315983
Exon 13	GTGACCTTGGAGAAGATATT	CTCTTGAAAGTTTACCAGCA	20316189–20316623
Exon 14	TTGCTCAAGGACTGAAATAG	CCTGCTTATCCTCAGAAGAG	20318223–20318732
Exon 15	GGTCTCATGGTCTCATTCTA	GGGTTTATCCTGCACTAGTA	20319650–20320025
Exon 16	AGAAGCACTTTGGGGTCTTGTA	GCTGAAATGGGAAGGAGAATC	20321144–20321581
Exon 17	GCTGAAAAACGATAGTCCAA	TCAACTAGATTACCCCTGTGT	20325354–20325863
Exons 18 and 19	TCACAGGGTGACAAGCAAC	TTGAATCTCTGGGTAGTTTG	20326820–20327678
Exon 20	GAAACCAGCAAGATCAGAGGA	TCACTCAGCTGGCATCAAAG	20338493–20338929
Exon 21	TGACTGTGACATCTGCTTGC	GGACAGAGGACATATTGCTCC	20338927–20339248
Exons 22 and 23	GCATTGTATTTCAGCATTGT	ACAGTGTTGTCTAGGGGGAC	20339701–20340506
Exon 24	GAACACACAGAATCCAACAGA	TCACTTCAGCTTCAGACAGT	20342562–20343001
Exon 25	TCTCATTGGTCTCCTCCTCG	AATTTCACACCACTAGCCAT	20344186–20344672
Exon 26	GAGGCATTGCCTAAGAGTGC	AAAGATGGAGCCAGGGTTTG	20350122–20350523
Exons 27 and 28	GGCAAGGATTGTCTTTCTTA	CGACAGCTGCGGTAAGTCTG	20351928–20352954
Exon 29	AGAGATGGAGTAGCCAGTCAC	CAGCCACAAATGCATATTACC	20353790–20354262
Exon 30	GAAGCTCAACCACAAACCAG	GCTCGACCAGTTTTCAAGAG	20355106–20355610
Exon 31	GCAAGGTACAGCTAGTTGAA	GCGTGATGTAAAATTTTGGC	20358730–20359248
Exon 32	GCTGTGGCTCATTGATTTTC	AAGGTGATAAAACAGAAATG	20359651–20360213
Sequencing			
5′-Flanking (for −1.7 k)	CCACCAGTGCCAAGAGAAGTAT	CACAAGTCATCTGGAAAACACA[b]	
(for −1.7 k to −1.3 k)	GGTATCTAACTGTGGTTTTG	GAAGGAAAGGAGTCAAAGGAAC	
(for −1.5 k to −950)	TCCCACACTGAATGCTGCCTTT	TAGGGACGGGGTCTCACTAT	
(for −880 to −400)	GGAAGATCGCTTGAACCCAT[b]	ATGTGCAGTTTCGCTTCTG	
(for −570 to −130)	CATATAGGCTCACACTGGAT	TCATCCCAACCATTTAATCG[b]	
Exon 1	TGGTTCCTTTTATGTATGGC	GTTCTTGTTGGTGACCACCC	
Exon 2	AAAGCAGTGGGATGTGCTG	TGTCTCTACTGTGCACCAAGG	
Exon 3	CACCGGAAACCATTCTGTTC[b]	TTTGCCTCACTATGGATCCC[b]	
Exon 4	CCTCCTTTCTTCCCATGTTC	CTCAACTTGATGCCATTTAC	
Exon 5	TGGGGCAACCTCTAACTCATA	TGAGACCCAGACATCTTAAA	
Exon 6	TTAGGGTCTCCAAATAAACA	ACTTTCAGAGGAGTGAGAGAGT	
Exon 7	GGTGGAGATAGCCTCTGACC[b]	TGCACTGAGAAGTATGAAGTGC[b]	
Exon 8	CCTGTACAGAGAAGGCCACG[b]	CACAATGCTGTAAGGTTAAG	
Exon 9	GGCTTTGGACAATTCTGGTC[b]	TCCACCCATTGTCTGTGAAC[b]	
Exon 10	GTGCCTTGGAGAAGCTGTGT	TTGCCCAAACTCCCATTAAG[b]	
Exon 11	TCACTGGGCACCTCAAGTTC	GGAATCCATCACCTCTACCA	
Exon 12	ACATTTTGGGGACTATATCT	ATGCCAGCTAGTCTATCAAA	
Exon 13	GGAGGCTGGATGATCCTTAAG	CTCTTGAAAGTTTACCAGCA[b]	
Exon 14	CATCTGTCTATGGTGGGATA	ATAGGCTCAAGACAAATCTC	
Exon 15	GATTTCATTCACCTCCTGTT	CATTTCCCCATGCATTCTAT	
Exon 16	CCAATCTTGAGGGGAAATCT	TCCAAGACCTCACCTACTAGC	

Table 1. continued

Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]
Exon 17	GTGGAATAACTACAAGCACG	TCAACTAGATTACCCCTGTGT[b]	
Exon 18	GGTGACAAGCAACAAAACTA	CCACCATCTTCCCTGTCTTA	
Exon 19	GATGCTCATGTAGGAAAACA	TTTACCATTCCACCCATGGC	
Exon 20	GGCTTCTCTCTCCTTGTTCA	CAAAGAAACAAAGGAAGAGC	
Exon 21	TGACTGTGACATCTGCTTGC[b]	GGACAGAGGACATATTGCTCC[b]	
Exon 22	GCATTGTATTTCAGCATTGT[b]	GATATTTGATGCATGGACGA	
Exon 23	GAATCTGTCTGGACCCTGTA	GTCTAGGGGGACATAATAAT	
Exon 24	ACACACAGAATCCAACAGAT	TCAACATATGACTAAATGGC	
Exon 25	GGAGCCTCTCATCATTCTGC	TTTCACACCACTAGCCATGC	
Exon 26	CCGATCAAGTCAAACCCTCT	TTTGAACCTCAGTCTTCTTT	
Exon 27	TTTCCTTACTCCCTTGTAGA	AAACTTTAGGGACCCATTAT	
Exon 28	CTGCTACCCTTCTCCTGTTC	CCTTCCCTCTGATACTGTGT	
Exon 29	TACCTCCTGTGACTGTGAAT	CAGCCACAAATGCATATTACC[b]	
Exon 30	GCCAGTCCTATCCACCATCT	AACACGAGGAACACGAGGAG	
Exon 31	GATCTGGAACATGAAAATGG	TTTTGGCCAGATTACTTGAC	
Exon 32	GCTCATTGATTTTCACTGCT	AAGGCAAAGGAATAATTATCG	

[a]The reference sequence is NT_030059.12.
[b]The same primer that was used for the 2nd PCR.

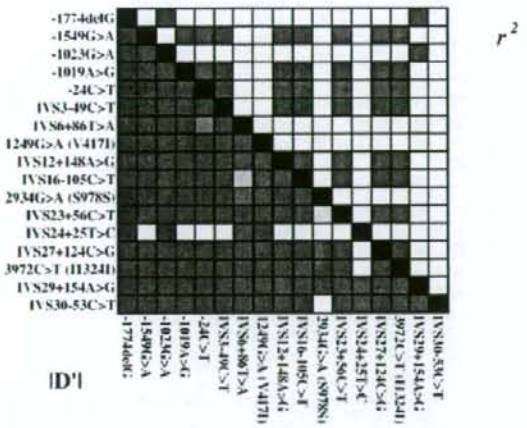

Fig. 1. Linkage disequilibrium (LD) analysis of *ABCC2*
Pairwise LD (r^2 values and |D′|) of polymorphisms detected in no less than 3% of allele frequencies is shown as a 10-graded blue color.

(**Table 2**). All detected variations were in Hardy-Weinberg equilibrium ($p > 0.05$). Novel variations consisted of 5 non-synonymous and 4 synonymous variations in the coding region, 22 in the intronic regions, 3 in the 5′-flanking region, 1 in the 3′- flanking region, and 1 in the 3′-UTR. The novel non-synonymous variations were 1177C>T (Arg393Trp), 1202A>G (Tyr401Cys), 2358C>A (Asp786Glu), 2801G>A (Arg934Gln), and 3320T>G (Leu1107Arg), and their frequencies were 0.002. No statistically significant differences were found in the allele frequencies of all variations between 177 cancer patients and 59 healthy subjects ($P > 0.05$, Fisher's exact test), although a larger number of subjects would be needed to conclude.

The frequency of the known common SNP −24C>T (0.173) was comparable to those reported in Asians (0.17–0.25)[8,12,20] and Caucasians (0.15–0.23)[9,10,14,15,21]. The allele frequency of another common SNP, 3972C>T (Ile1324Ile) (0.216), was also comparable to those in Asians (0.22–0.30)[8,12,20] but lower than those in Caucasians (0.32–0.37)[9,10,14,15,21]. The other major variations in the 5′-flanking region, −1774delG and −1549G>A, were found at frequencies of 0.343 and 0.203, respectively, and these values were similar to those obtained in Koreans (0.34 and 0.21, respectively).[8] However, the relatively frequent SNPs 1446C>G (Thr482Thr) (allele frequency=0.125), IVS15-28C>A (0.333) and IVS28+16G>A (0.167) in Caucasians[17] were not detected in our study.

The LD profile of the *ABCC* variations (no less than 3% allele frequency) is shown in **Figure 1**. As assessed by r^2 values, close linkages were observed among −1774delG, −1023G>A and IVS29+154A>G, and among −1549G>A, −1019A>G, −24C>T, IVS3-49C>T, IVS12+148A>G, IVS15+169T>C, IVS16-105C>T, IVA23+56C>T, IVS27+124C>G, and 3972C>T (Ile1324Ile). It must be noted that complete linkage was observed between −1549G>A and −1019A>G in our population. In |D′| values, strong LD was also observed almost throughout the region analyzed. Overall, since close associations between the variations were observed throughout the entire *ABCC2* gene, the region sequenced was analyzed as a single LD block for the haplotype inference.

The *ABCC2* haplotype structures were analyzed using 61 detected genetic variations and a total of 64 haplotypes were identified/inferred. **Figure 2** summarizes the haplotypes and their grouping. Our nomenclature system is based on the recommendation of Nebert.[22] Haplotypes without