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SNP Genotype in *ABCB1* is Associated with Completion of Intraperitoneal Chemotherapy in Ovarian and Primary Peritoneal Cancer.

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Sergio Enderica González
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Dedicatoria

A ustedes Tío Justo, Mamá y Tita por siempre estar a mi lado.

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Resumen

Introducción: El tratamiento estándar para la terapia neo-adyuvante del Cáncer de Peritoneal Primario (PP) y de Ovario (OC) se basa en quimioterapia basadas en platino administradas por vía intravenosa (IV) o intraperitoneal, pero esta última está asociada a mayor cantidad de efectos adversos grado 3/4. Para identificar marcadores genéticos que estén asociados a completar los ciclos de quimioterapia intraperitoneal, nosotros analizamos polimorfismos de nucleótidos simple (SNP) en 4 genes involucrados en el metabolismo de las drogas basadas en platino o taxanos.
Métodos: Pacientes diagnosticados con cáncer epitelial de ovario o peritoneal primario que sean recurrentes o primarios, que hayan sido óptimamente extirpados y que hayan sido tratados con quimioterapia IV o IP en la Clínica Mayo (Rochester, MN) entre el periodo de junio del 2007 hasta febrero del 2009 fueron reclutados. Se analizó el ADN germinal usando un panel personalizado de Ilumina BeadXpress 96-plex. Los genotipos en los SNPs en GSTM1 (N=7), ABCB1 (N=57), CYP3A4 (N=7), and CYP2C8 (N=25) fueron correlacionados con completar seis ciclos de quimioterapia IP usando regresión logística.
Resultados: Treinta y siete pacientes fueron incluidos y 16 (43.2%) completaron seis ciclos de quimioterapia intraperitoneal. Veintidós de 57 SNPs en el gen ABCB1 fueron asociados con completar todos los ciclos ($p<0.15$), pero el alelo menor A en el rs2229109(missense) llego a tener la mayor significancia estadística presentándose en 5 (31.2%) de los pacientes que terminaron todos los ciclos de quimioterapia IP, y en solo uno (4.8%) que no completo. Es importante recalcar que este último paciente no completo por complicaciones relacionadas al catéter. No se encontraron asociaciones significativas en los SNPs de los genes GSTM1. CYP3A4, y CYP2C8. No se encontró asociación entre rs2229109 y sobrevivencia.
Conclusiones: Nuestros resultados sugieren que el SNP rs2229109 (G1199A) en el gen ABCB1 este asociado con la terminación de los seis ciclos de quimioterapia IP, y potencialmente menos toxicidad. Estos pacientes con serían los candidatos ideales para recibir quimioterapia IP, pero se necesitan más estudios.

Palabras Claves: Cisplatino, Paclitaxel, ABCB1, Cáncer de Ovario, Quimioterapia Intraperitoneal.

ABSTRACT

Introduction: A standard of care (SOC) for adjuvant ovarian (OC) and primary peritoneal (PP) cancer treatment is a platinum-based intravenous (IV) or intraperitoneal (IP) chemotherapy regimen, but the latter is associated with more frequent grade 3/4 adverse events. To identify genetic markers for completion of IP chemotherapy, we analyzed SNPs in four genes, selected for their involvement in metabolism of platinum or taxane drugs.
Methods: Patients who had primary/recurrent stage III/IV optimally debulked epithelial OC or PP cancers treated with adjuvant IP and IV chemotherapy at Mayo Clinic (Rochester, MN) between January 2007 and February 2009. Germline DNA was analyzed using a custom Illumina BeadXpress 96-plex panel. SNPs in GSTM1 (N=7), ABCB1 (N=57), CYP3A4 (N=7), and CYP2C8 (N=25) were genotyped and correlated with completion of chemotherapy cycles by linear regression.
Results: Thirty-seven patients were included and 16 (43.2%) completed six IP chemotherapy cycles. Twenty-two of 57 ABCB1 SNPs were associated with completion ($p<0.15$) but the minor A allele at rs2229109 (missense) reached the highest significance: present in five (31.2%) who completed treatment and only one (4.8%) who did not ($p=0.007$). However, the latter was due to catheter complications. No significant associations were observed for GSTM1. CYP3A4, and CYP2C8 SNPs. There were no associations between rs2229109 and overall or progression-free survival.
Conclusion: Our findings suggest that SNP rs2229109 (G1199A) in ABCB1 gene is associated with IP chemotherapy completion, and potentially, less toxicity. These patients may be optimal candidates for IP chemotherapy and further studies are warranted.

Keywords: Cisplatin, Paclitaxel, ABCB1, Ovarian Cancer, Intraperitoneal Chemotherapy.

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Introduction

Ovarian cancer is the most lethal gynecological malignancy, accounting for 22, 280 new cases and 15,500 deaths in 2012[1]. Standard treatment of advanced ovarian cancer involves cytoreductive surgery followed by a platinum- and taxane-based chemotherapy doublet. Despite advances in chemotherapy combinations and schedules, the majority will eventually relapse and die from recurrent disease, with five-year survival rates approximately 30-40%[2, 3].

The pattern of intraperitoneal dissemination seen in patients with ovarian, primary peritoneal, and fallopian tube cancers led to the development of intraperitoneal (IP) chemotherapy regimens to improve outcomes. IP-containing regimens are associated with significant survival benefits over strictly IV chemotherapy [4]. However, the higher incidence of grade 3 and 4 adverse effects have impeded acceptance of IP regimens into clinical practice. Identifying predictors of toxicity could assist with patient-selection.

Single nucleotide polymorphisms (SNPs) have been useful in identifying genotypes that are associated with metabolism, transportation, and activity of chemotherapy and may contribute to differences in therapeutic efficacy and severity of adverse events [5-7]. For instance, the intracellular disposition of paclitaxel can be altered by p-glycoprotein pump (P-gp), a transmembrane ATP-dependent drug efflux pump and member of the ATP-binding cassette (ABC) transporter superfamily. It is encoded by *ABCB1* (also known as *MDR1*). Although polymorphisms in *ABCB1* are associated with survival [8, 9], presumably through mechanisms involved in chemotherapy resistance, less is known about the association between such SNPs and treatment toxicity. For example, a C3435T or G2677T/A genotype is associated with more pronounced

neutrophil decline from baseline [5, 10], suggesting greater myelotoxicity in these patients, but there was no increased risk of severe febrile neutropenia. Although C3435T was associated with a lower incidence of peripheral neuropathy [10], these findings were not supported by larger studies where no association between neurotoxicity or neutropenia was seen in the SCOTROC1 trial (n=914) or in a retrospective analysis of AGO-OVAR-9 and NSGO-OC9804 (n=119) [11, 12]. Although the data for genetic polymorphisms and chemotherapy toxicity in ovarian cancer patients treated with IV chemotherapy is mixed, no such studies have been performed for patients receiving IP chemotherapy, where adverse events are more common [4, 13-18].

Cytochrome P450 enzymes, primarily *CYP2C8* and *CYP3A4*, play an important role in paclitaxel metabolism and alterations of enzymatic activity could affect drug levels (including metabolites) and thus, influence toxicity. The relationship between genetic polymorphisms in ovarian cancer patients and IV chemotherapy-related toxicities is also unclear. For instance, *CYP2C8*3* was initially associated with an 11% reduction of the paclitaxel clearance [19] and increased risk of neuropathy [20] while *CYP2C8-HapC* was associated with treatment-related leukopenia and neutropenia [21]. However, larger studies found no association between *CYP2C8* or *CYP3A4* and increased risk for such toxicities [11, 12].

Glutathione S transferase (GST) enzymes are comprised of eight distinct classes: alpha, kappa, mu, omega, pi, sigma, theta and zeta. *GSTM1* encodes a GST belonging to the mu class and detoxifies electrophilic compounds such as cisplatin. Although the *GSTM1*-null genotype has been studied in relation to survival [8, 22-25], less is known about the role of *GSTM1* regarding chemotherapy toxicity. In 104 ovarian cancer patient treated with cisplatin-cyclophosphamide, the *GSTM1*-null genotype was associated with a lower risk of cytopenia and neuropathy relative to the wild-type genotype [6].

In light of the discrepant data regarding SNPs and chemotherapy toxicities in ovarian cancer patients treated with IV chemotherapy and the paucity of similar data for IP chemotherapy, we investigated the association between SNPs encoded by common carboplatin- or paclitaxel-metabolizing genes. We hypothesized that single nucleotide polymorphism in these genes may predict tolerability of six IP cycles of chemotherapy and be a useful indicator of toxicity. Therefore, we analyzed 96 SNPs in *ABCB1, CYP2C8, CYP3A4* and *GSTM1* from 37 patients receiving intraperitoneal chemotherapy for epithelial ovarian or primary peritoneal cancer.

Methods

Patient Population

Patients with primary or recurrent epithelial ovarian or primary peritoneal cancer undergoing primary or secondary debulking surgery at the Mayo Clinic (Rochester, MN) between January of 2007 and February of 2009 were eligible for this study. All patients received at least one cycle of intraperitoneal chemotherapy. A cycle was defined as IV paclitaxel (135mg/m^2) on day one, IP cisplatin (100mg/m^2) on day two, and IP paclitaxel (60mg/ m^2) on day eight. For patients to be defined as a completing intraperitoneal chemotherapy, they should have finished six cycles of this regimen at full or reduced doses. Patients that had five cycles or less of IP chemotherapy were classified as incomplete. Histologic features, surgical outcomes, and recurrence status of each patient was abstracted via the electronic medical record system in accordance with the Mayo Clinic Institutional Review Board

DNA extraction and genotyping

Patient germline DNA was extracted from pre-treatment peripheral blood using the Gentra Autopure LS Purgene (Gentra, Minneapolis,MN) salting out method. Samples were barcoded to

protect patient privacy and stored at a - 20°C. A custom Illumina BeadXpress 96-plex panel was used to genotype single nucleotide polymorphism in *GSTM1* (N=7), *ABCB1* (N=57), *CYP3A4* (N=7), and *CYP2C8* (N=25).

SNP Selection

To create ld tagSNPs for the 4 candidate genes, we used genotypes from the the genome-wide genotyping projects Hapmap Phase II (http://www.hapmap.org) and Perlegen (www.perlegen.com - URL no longer valid as company is out of business). We also used genotypes from the gene resequencing programs: Seattle SNPs (http://pga.mbt.washington.edu/) and NIEHS SNPs (http://egp.gs.washington.edu/). To determine the Hapmap and Perlegen SNPs for each of the candidate genes, we picked SNPs 5kb upstream and 5 kb downstream of each gene. Our gene and SNP coordinates were based on RefSeq release 29 (NCBI build 36), and dbSNP build 129. If the gene had been resequenced in Seattle SNPs or NIEHS SNPs, we used genotypes from those sources as well.

To pick ld tagSNPs, we ran ldSelect [26] on each gene for each genotype source (Hapmap, Perlegen, Seattle, NIEHS) for the Caucasian samples in those public sources. We used an r^2 of 0.95 and a minor allele frequency (maf) cutoff of 0.05. To determine the best source of genotypes for each gene, we chose the source with the higher number of ld bins for the Caucasian samples after bins were removed that didn't have a tagSNP with an assay score of 0.4 or greater. If 2 sources (e.g. Hapmap, Seattle SNPs) had the same number of bins, we used Hapmap as the best source because of its higher number of samples (60 unrelated Caucasian samples). Hapmap was chosen as best source for 3 of the genes and NIEHS was chosen for CYP3A4. To pick the best tagSNP for each LD bin, we used the SNPPicker software [27] LdSelect often gives multiple choices of tagSNPs for a given bins but not all tagSNPs have the same design probability or

possible functional relevance. SNPPicker picks the best tagSNP for each bin, optimizing constraints such as assay score and functional relevance. It also allows multiple tagSNPs for bins. To reduce the probability of failure, we picked 2 tagSNPs where possible while 3 were chosen for bins with size of 30 or greater. All tagSNPs met the minimum Illumina assay score of 0.4.

In addition to tagSNPs, we included potentially functional SNPs that had not been already chosen as tagSNPs. This included missense SNPs with maf (as reported by Illumina) in a Caucasian population > 0. We also included synonymous, 5' upstream (within 5kb of candidate gene), 5' UTR, 3' UTR, 3' downstream (within 5kb of candidate gene) SNPs with maf >= 5%

Statistical analysis

Association between SNPs and the number of cycles of chemotherapy was performed with linear regression using an additive model for each SNP.

Results

Thirty-seven patients diagnosed with ovarian or primary peritoneal cancer were included in this study and successfully genotyped. Patient characteristics are shown in Table 1. The majority of patients were diagnosed with ovarian cancer (75.6%) and the remaining with primary peritoneal cancer (24.3%). The most common histological subtype was serous (78.2%), followed by endometrioid and clear cell. All patients underwent primary or secondary optimal surgical debulking at Mayo Clinic between January of 2007 to February of 2009. Till June of 2012, 56.7% of the thirty-seven patients have recurred, 37.8% are disease free, and 5.4% are lost to follow-up. Sixteen patients (43.2%) out of the thirty-seven patients enrolled in this study were able to complete the six cycles of IP chemotherapy, while twenty-one (56.7%) had to abandon this

regimen due to adverse events. *ABCB1, GSTM1, CYP2C8* and *CYP3A4* were genotyped for SNPs associated with completion of IP chemotherapy (Table 2). SNPs in *ABCB1* demonstrated statistically significant associations with the number of chemotherapy cycles. The minor allele G1199A (S400N) at rs2229109 was significantly associated with completion of the six cycles of IP chemotherapy (p=0.007) and was present in 5 of 16 patients (31.2%) completing IP chemotherapy (Table 3). Only one patient with G1199A was unable to complete IP chemotherapy but this was attributed to catheter infection rather than chemotherapy toxicity.

Other *ABCB1* SNPs associated with the number of intraperitoneal chemotherapy cycles were intronic: rs17327442 (p=0.028), rs6961665 (p= 0.093), rs13233308 (p=0.093) and rs4148732 (p=0.062)(Table 2). These SNPs are close to each other and to rs2229109 in *ABCB1* (Figure 1). Out of the 57 SNPs analyzed in *ABCB1*, 22 SNPs also had some degree of association with the number of chemotherapy cycles ($p<0.15$) (Table 4 and Figure 1). No association between rs2229109 (G1199A) and progression free survival or overall survival was seen in this study.

No statistically significant association was seen between *GSTM1, CYP3A4* and *CYP2C8* and the number of intraperitoneal chemotherapy cycles.

Discussion

To our knowledge, this is the first study to report the association of single nucleotide polymorphisms in key genes involved in the transport and metabolism of platinum – taxane chemotherapies in ovarian/primary peritoneal cancer patients treated with intraperitoneal chemotherapy. Ninety-six SNPs in four different genes involved in the metabolism of platinum and taxane agents were genotyped from thirty-seven patients. Results showed that a minor A allele (G1199A) at SNP rs2229109 in *ABCB1* was more likely to complete all cycles of intraperitoneal

chemotherapy than those who did not carry this SNP (p=0.007). We could not find any association between *CYP3A4*, *CYP2C8* and *GSTM1* and completion of chemotherapy.

Although larger studies have not shown an association between *ABCB1* polymorphisms and IV chemotherapy-related toxicity, the different pharmacokinetics of IP chemotherapy may obviate the applicability of these data to patients presented herein. Indeed, the IP route achieves much higher peritoneal-to-plasma AUC ratios: carboplatin (12-fold), cisplatin (10-18 fold), and paclitaxel (1000-fold) [28-30]. Since expression of *ABCB1* is high in normal gastrointestinal (liver and bowel lumen) tissues [31-33], drainage of fluid from the visceral peritoneum into the portal vein subjects xenobiotics to hepatic first-pass metabolism [34] and altered function of this gene could impact systemic bioavailability and toxicity. Indeed, G1199A is a missense allele that, when expressed in recombinant epithelial cells (ABCB1-1199A), confers greater ability to efflux fluorescently tagged substrates in the basal-to-apical direction [35]. This phenomenon is substrate-specific since vincristine and vinblastine, but not doxorubicin, exhibit greater permeability in ABCB1-1199A cells [36]. Although the permeability of paclitaxel was not directly studied, ABCB1-1199A cells were more resistant to paclitaxel, suggesting G1199A alters permeability of paclitaxel in normal cells and may alter bioavailability. Thus, G1199A (rs2229109) may be associated with completion of IP chemotherapy cycles by virtue of lower systemic chemotherapy levels and thus, less toxicity. Although there was no association with survival, these data are consistent with larger studies [37]. Although no associations were seen with *GSTM1*, *CYP2C8* and *CYP3A4* polymorphisms and completion of IP chemotherapy, these data are consistent with larger studies showing no increased risk for toxicities [11, 12]. The absence of significant association between these genes and completion of IP platinum/taxane chemotherapy may reflect a lack of understanding of the intracellular mechanisms of metabolism of these compounds via the intraperitoneal route.

Intraperitoneal platinum-taxane chemotherapy for ovarian, primary peritoneal, and fallopian tube cancer has demonstrated improved survival over IV-alone chemotherapy and on January 5, 2006, the National Cancer Institute (NCI) announced a recommendation to consider combination IV/IP chemotherapy regimens for all optimally debulked patients with good performance status [38]. However, a survey of surgical and medical oncologists suggests the clinical practice has been slow to implement such therapy[39]. Although 77% of respondents discussed IP chemotherapy with patients, survey response rates were low among surgical (24%) and medical (3%) oncologists; as such, the true frequency of IP-chemotherapy counseling may be much lower than reported. One of the main reasons is catheter-related complications but also, higher incidences of hematologic and gastrointestinal adverse events have been a hindrance. For these reasons, an international consensus did not support making IP chemotherapy the new standard of care [40]. Predictors of IP chemotherapy tolerability may enable clinicians to select optimal patients for treatment.

In conclusion, this study provides the first evidence that single nucleotide polymorphisms in *ABCB1* are associated with completion of the six cycles of intraperitoneal chemotherapy, and possibly less serious adverse events for this regimen. These findings will need to be reproduced in a larger population set, preferably with case-control or matched cohort designs to eliminate confounders and allow us to assess whether SNP rs2229109 impacts clinical outcomes. This knowledge will help us determine which subset of patients will be the most suitable candidates for intraperitoneal chemotherapy.

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TABLES

Table 1. - Patient Characteristic.

	Patients (N)	**(%)**
Age (years)		
<50	6	16.2
50-59	8	21.6
60-69	14	37.8
>70	9	24.3
Primary Tumor Site		
Ovary	28	75.6
Peritoneal	9	24.3
Histological Subtypes		
Serous	29	78.2
Endometrioid	6	16.2
Clear Cell	2	5.4
Post-Surgery tumor volume		
Optimal	37	100
Suboptimal	0	0
No. of Cycles of IP Chemotherapy		
6 cycles	16	43.2
<5 cycles	21	56.7
Recurrence Status		
Recurred or deceased	21	56.7
No recurrence	14	37.8
Unknown	2	5.4

Table 2. - List of genes analyzed.

Name	Chr	GeneID	Description	SNPs (N)	P<0.05*
ABCB1	7	5243	ATP-binding cassette, sub-family B, member 1	57	Yes
CYP2C8	10	1558	Cytochrome P450, family 2, subfamily C, polypeptide 8	25	No
CYP3A4	7	1576	Cytochrome P450, family 3, subfamily A, polypeptide 4	7	No
GSTM1	1	2944	Glutathione S Transferase M1	7	No

*P values reflect SNP level significance.

Table 3. - *ABCB1* [rs2229109] genotype in our patient population.

Allele	No. Of Cycles	N (%)	Total N (%)
G/A	6	5 (83.3)	
	≤5	1 (16.6)	
			6 (16.2%)
G/G	6	11 (35.4)	
	≤5	20 (64.5)	
			31 (83.7%)

Table 4. - Most significant SNPs associated with completion of IP chemotherapy.

Gene	RSID	P Value	Base Substitution	Variant Type
ABCB1	RS2229109	0.007	A/G	missense
	RS6961665	0.093	A/C	intron
	RS17327442	0.028	A/T	intron
	RS4148732	0.063	A/G	intron
	RS13233308	0.093	C/T	intron
	RS998671	0.802	A/G	3'downstream
	RS6946119	1	C/T	3'downstream
	RS7802783	0.802	C/T	3'downstream
	RS1055302	0.802	A/G	3'downstream
	RS3842	0.802	A/G	3'UTR

RS17064	0.916	A/T	3'UTR
RS2235048	0.154	C/T	intron
RS1045642	0.154	A/C/T	synonymous
RS6949448	0.391	C/T	intron
RS2373589	0.96	A/G	intron
RS7787082	0.96	A/G	intron
RS2032581	-	A/G	missense
RS11983225	0.744	C/T	intron
RS11760837	0.744	C/T	intron
RS12720066	0.582	G/T	intron
RS4148737	0.443	A/G	intron
RS4728700	0.129	C/T	intron
RS1922242	0.443	A/T	intron
RS2235046	0.129	A/G	intron
RS2091766	0.443	C/T	intron
RS2235033	0.093	C/T	intron
RS2032588	0.258	C/T	intron
RS1128503	0.129	C/T	synonymous
RS3789244	0.129	A/C	intron
RS1922240	0.259	C/T	intron
RS1922241	0.259	A/G	intron
RS2235023	0.258	A/G	intron
RS4148734	0.259	C/T	intron
RS1202169	0.129	A/G	intron
RS6950978	0.259	A/T	intron
RS12334183	0.337	C/T	intron
RS10260862	-	C/G	intron
RS10264990	0.107	C/T	intron
RS1989830	0.891	C/T	intron
RS13226726	0.666	C/T	intron
RS1202172	0.891	G/T	intron
RS1202184	0.203	A/G	intron
RS1211152	0.969	G/T	intron
RS17327624	0.161	G/T	intron
RS13229143	0.627	C/G	intron
RS12535512	0.528	C/T	intron
RS2188526	0.5	A/G	intron
RS3789243	0.369	C/T	intron
RS1858923	0.5	C/T	intron
RS2888599	0.43	A/G	intron
RS9282564	0.835	A/G	missense
RS2214102	0.993	A/G	5'UTR
RS3213619	0.43	C/T	5'UTR

RS4728709	0.326	A/G	intron
RS10246878	0.526	A/G	intron
RS10267099	0.526	A/G	intron
RS6972098	0.326	C/T	5'upstream

FIGURES

Figure 1.-

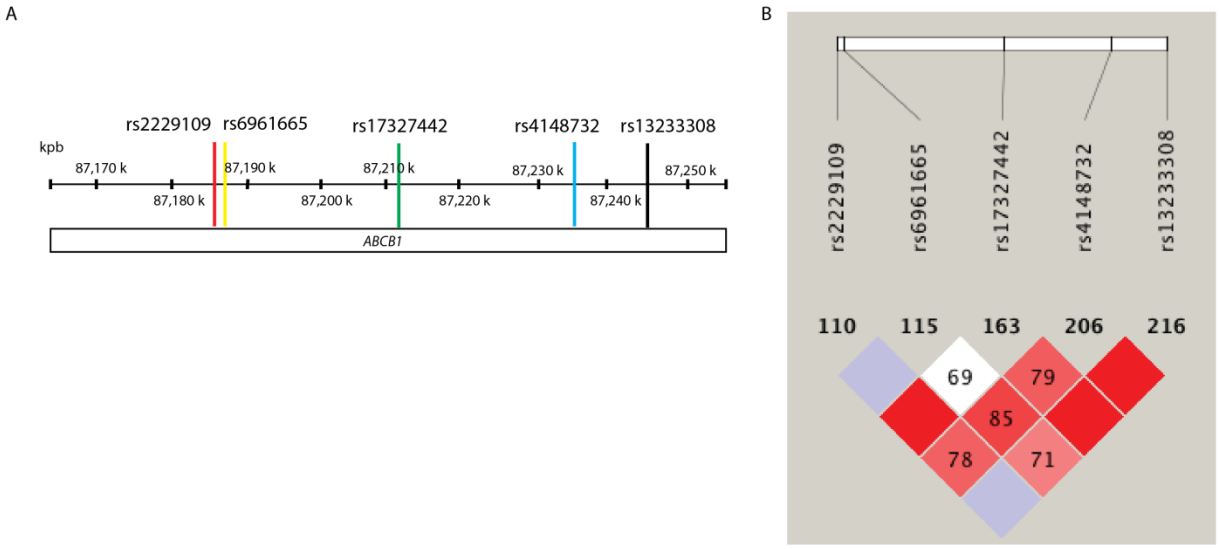

Figure 1. a) *ABCB1* is located on chromosome 7 in the q21.12 region. The five most significant SNPs associated with completion of IP chemotherapy, rs2229109, rs6961665, rs17327442, rs4148732 and rs13233308, are located 1.6kpb, 31.5kpb, 21 kpb and 10.9 kpb away from each other, respectively. b) Linkage Disequilibrium plot of *ABCB1* was created using Haploview v4.2. Standard color scheme: white =(D′<1, LOD<2), blue =(D′=1, DOD<2), shades of pink/red =(D′<1, LOD ≥ 2), bright red =(D′=1, LOD ≥2). The numbers inside the squares represent the D′ values.