Identification of Polymorphisms Associated with Hypertriglyceridemia and Prolonged Survival Induced by Bexarotene in Treating Non-small Cell Lung Cancer

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Abstract. *Background: Bexarotene was evaluated in treating advanced non small cell lung cancer (NSCLC) in two phase III trials. Although a significant survival benefit was not observed for the overall bexarotene-treated population (617 patients), a third of bexarotene-treated patients who developed high-grade hypertriglyceridemia exhibited significantly longer survival. Patients and Methods: In order to identify genomic polymorphisms that could serve as potential predictive biomarkers for response and improved survival in NSCLC patients, DNA samples extracted from plasma archived from 403 patients were genotyped using Affymetrix 500K whole genome SNP arrays and/or Sequenom iPLEX™ assays. Results: Fourteen SNPs were identified on nine loci that showed significant associations with high-grade hypertriglyceridemia induced by bexarotene. Four such single nucleotide polymorphisms (SNPs) reside on the region upstream of solute carrier family 10, member 2 (SLC10A2), and one SNP is located close to lymphocyte cytosolic protein 1 (LCP1), whose expression correlated with the activity of bexarotene in tumor cells. Conclusion: We identified novel polymorphisms exhibiting significant association with bexarotene induced hypertriglyceridemia, implicating their potential in predicting bexarotene-improved survival response.*

Bexarotene (also called Targretin, LGD1069) is a selective modulator of retinoid X receptors (RXRs) approved for treating refractory advanced-stage cutaneous T-cell lymphoma (1, 2). A number of preclinical studies and phase I and II clinical trials have shown that bexarotene also exhibits promising antitumor or tumor prevention activity for breast cancer, renal cell carcinoma and lung cancer (3-8). Consequently, two large phase III trials (SPIRIT I and SPIRIT II) were conducted to evaluate the efficacy and safety of standard chemotherapy agents with or without bexarotene as a first-line therapy in treating advanced NSCLC. The results from both phase III trials, however, showed that the addition of bexarotene to chemotherapy did not improve overall survival in the intent-to-treat population, the primary efficacy endpoint (9, 10). A known side-effect of retinoid therapy is the elevation of serum lipids, and the majority of the bexarotene treated patients in the two SPIRIT trials developed hypertriglyceridemia, as expected. Further analysis revealed that 30-40% of the patients appeared to be more sensitive to bexarotene treatment and developed NCI grade 3 or higher hypertriglyceridemia. Survival analysis in this subgroup of patients in each of the two trials revealed significantly longer survival compared to the patients in the control arm and to patients with low-grade hypertriglyceridemia (9, 10). This intriguing correlation between survival and triglyceride level induced by bexarotene observed in both SPIRIT trials is illustrated in Figure 1A. Similar correlations were also revealed by retrospective analysis in other bexarotene cancer trials (11). These findings prompted the search for biomarkers which can predict bexarotene sensitivity and identify a subgroup of NSCLC patients whose survival could be prolonged by bexarotene treatment. The molecular mechanism underlying the rexinoid-induced hypertriglyceridemia remains largely unknown, but the varied responses exhibited by the patients within 2-4 weeks of bexarotene treatment suggests this might be due to inherent biochemical and genetic differences among the patients. Therefore, a pharmacogenomic study was performed to identify genetic variations associated with bexarotene-induced hypertriglyceridemia using patient samples from the two SPIRIT trials.

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Patients and Methods

Patients. Among patients enrolled in the SPIRIT I and II trials and treated with bexarotene, plasma samples from 403 individuals were available. The clinical characteristics of these subjects are provided in Table I. The cases were defined as those whose triglyceride level percentiles were higher than 62 (upper third HTG Hi), and the controls were those whose triglyceride level percentiles were lower than 31 (lower third or HTG Lo). Those patients whose plasma triglyceride levels were between 31 and 62 were categorized into a middle group (HTG Mid).

DNA preparation. DNA was extracted from plasma samples with the QIAGEN QIAamp MinElute Virus Spin Kit (Valencia, CA, USA). DNA samples were amplified using the Amersham Bioscience GenomiPhi DNA Amplification Kit (Piscataway, NJ, USA).

SNP genotyping and data analysis. In Stage I (Figure 1B), 150 samples were genotyped using the Affymetrix GeneChip 500K Mapping Array Set containing 500,000 SNPs following Affymetrix standard protocol (Santa Clara, CA, USA). From the genome-wide scan results, 255 SNPs were selected for Stage II study using Sequenom iPLEX™ assays (Sequenom, San Diego, CA, USA). These assays were used to genotype 400 DNA samples from the SPIRIT trials. Among them, 147 samples out of the 150 samples used in Stage I were selected as the verification group, and the other 253 samples were used as the replication group (Figure 1B). Final genotype calls were generated by Sequenom Typer Analyzer from the MassARRAY Typer suite (Sequenom, San Diego, CA, USA). After removing the samples having call rates lower than 50%, 385 samples remained (141 samples in the verification group and 244 in the replication group). The case control analysis of associated SNPs was carried out using chi-square test based on differences in allele counts between cases and controls. Subsequently, the level of triglyceride percentile was used as a quantitative trait and association analysis was performed by least squares regression using PLINK program (12).

Paclitaxel-resistant MDA-MB-231 cells and resensitization with bexarotene treatment. The human breast cancer cell line MDA-MB-231 was obtained from the American Type Culture Collection (Manassas, VA, USA), and MDA-MB-231cells were routinely cultured in RPMI-1640 supplemented with 10% fetal bovine serum and 2 mM glutamine in 5% CO_2. Bexarotene was synthesized at Ligand Pharmaceuticals Inc. (San Diego, CA, USA), and paclitaxel was purchased from Sigma Chemicals (St. Louis, MO, USA). The protocol for establishment of paclitaxel-resistant MDA-MB-231 cells and resensitization to paclitaxel by bexarotene treatment was described previously (5). Briefly, MDA-MB-231 cells were exposed to regimens on a 10-day cycle: a 3-day treatment with 30 nM paclitaxel followed by a 7-day exposure to control medium. Paclitaxel-resistant MDA-MB-231 cells (MDA-PR) were established within 8 cycles of such treatment (3 days on and 7 days off; total of 80 days). These MDA-PR cells were then treated with the combination of 30 nM paclitaxel (3 days on and 7 days off) in the absence or presence of 1 μM Targretin (10 days on) in a new 10-day cycle for 3 months. MDA-PR cells thus treated became re-sensitized to paclitaxel. Meanwhile, the regimen including paclitaxel alone had no effect on the growth of MDA-PR cells.

Table I. *Baseline characteristics by triglyceride subgroup*.

Parameter	In-treatment triglyceride subgroups		
	Low (n=124)	Middle (n=129)	High (n=150)
Male (%)	75.8	70.5	64.7
Age (% in category)			
≤60 years	42.7	34.9	46.0
>60 years	57.3	65.1	54.0
Race (%)			
Non-White	8.9	8.5	5.3
White	91.1	91.5	94.7
Region (%)			
Australia	0.8	3.9	2.0
Eastern Europe	30.6	32.6	29.3
North America	33.9	32.6	40.0
South America	8.1	5.4	4.7
Western Europe/Israel	26.6	25.6	24.0
Mean baseline triglyceride (in percentile*)	38.3	47.2	61.4

*Percentiles of baseline triglyceride were calculated among all patients regardless of subgroup.

RNA microarray. RNA was extracted from parent and paclitaxel-resistant MDA-MB-231 breast cancer cells treated with vehicle control, paclitaxel alone or with bexarotene as described in previous section (Paclitaxel resistant MDA-MB-231 cells and resensitization with bexarotene treatment). All RNA samples had satisfactory 28S/18S ratio when analyzed with 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA). A single probe was prepared for each RNA sample and hybridized to a single array using the standard protocol and reagents supplied by Affymetrix, and the probe was then hybridized to Affymetrix HG-U133A array (22,283 probe sets). The microarray data and biological annotations were deposited at the GEO microarray repository (http://www.ncbi.nlm.nih.gov/geo) under GSE12791.

Results

Plasma samples from 403 patients who participated in the SPIRIT I and II trials were available for our study, and they were divided into high, middle and low groups based on their percentile of maximum triglyceride level exhibited during bexarotene treatment (Table I). This grouping strategy mimicked the grouping method illustrated in the survival analysis reported earlier (9, 10) and shown in Figure 1A, in which distinct survival results were observed when patients were divided into three groups based on their absolute triglyceride levels reached following bexarotene treatment.

In Stage I, 74 samples were randomly selected from the high HTG (case) and 76 samples were selected from the low HTG (control) subgroups, and they were genotyped

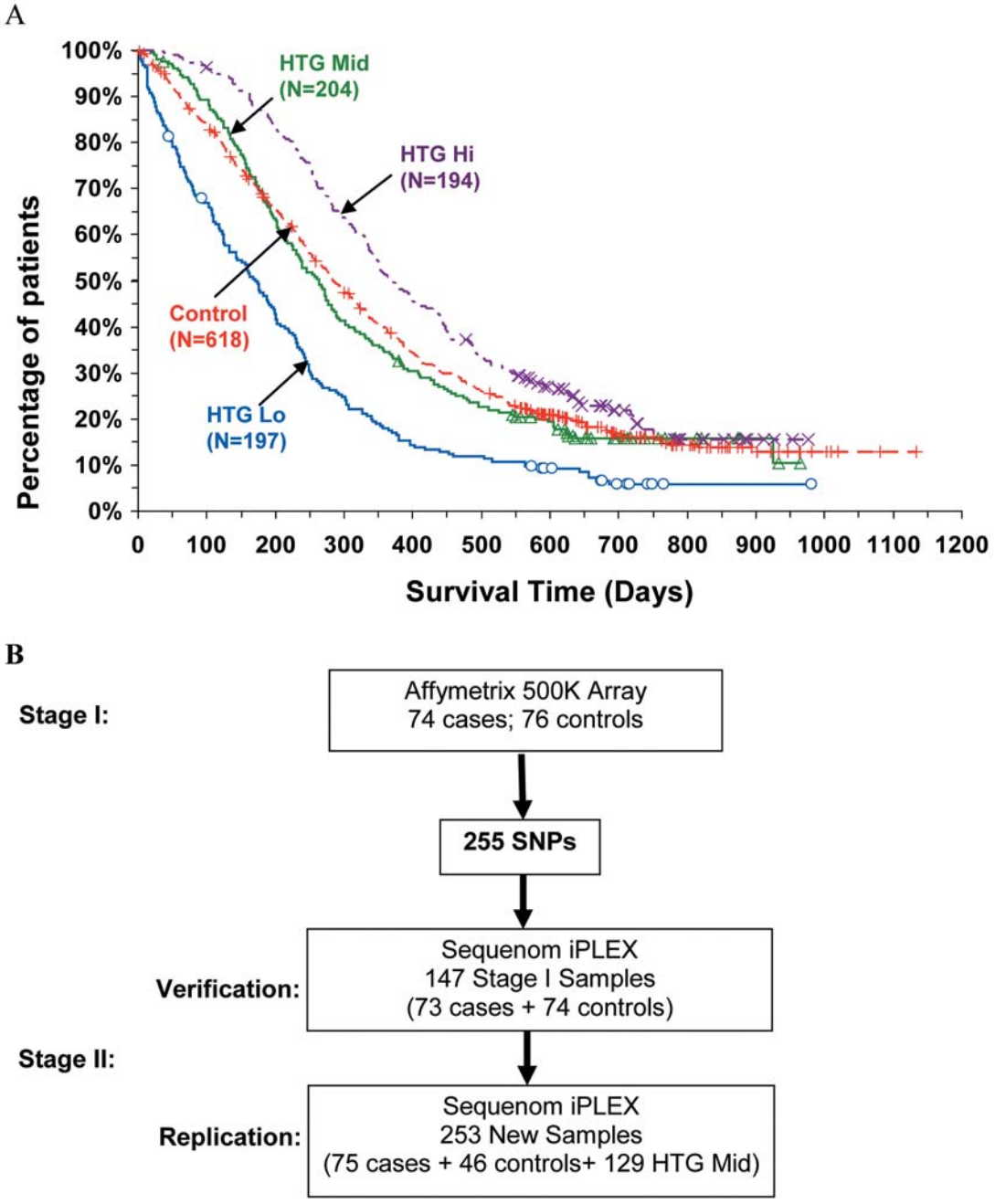

Figure 1. *Sub-population analysis and study design. A: Survival analysis of patients exhibiting different triglyceride levels following bexarotene treatment. The total number of patients (N=1213) included in the analysis were from both SPIRIT I and II studies, and they were divided into three subgroups based on the percentile of maximum triglyceride level they exhibited during the study. Therefore, 'HTG Lo' group includes those patients who experienced triglyceride levels up to 33% of the maximum triglyceride level in all treated patients, 'HTG Mid" includes the middle third (33% to 67%), and the 'HTG Hi' includes the patients exhibiting the highest levels of triglyceride (67% to maximum) during the treatment. Kaplan-Meier survival estimates in patients gave median survivals of 170 days, 263 days, and 371 days for these three groups, respectively. By this analysis, the control arm has a median survival of 284 days, which is significantly different from both the 'HTG Hi' group (log-rank p-value=0.003) and the 'HTG Lo' group (log-rank p-value <0.0001), but not from the 'HTG Mid' group (log-rank p-value=0.43). B: Overview of genome-wide association study design. In Stage I discovery phase, 150 samples (74 cases and 76 controls) were genotyped using the Affymetrix GeneChip 500K Mapping Array Set (Nsp array + Sty array) containing 500,000 SNPs following the Affymetrix standard protocol. 255 SNPs were then selected for stage II study. In Stage II, 400 samples were individually genotyped using the 255 Sequenom iPLEX SNP assays, including 147 samples (minus one case and two controls) used in the Stage I for the Verification group and 253 new samples for the Replication group. In the Replication group, 129 samples were included from the patients with middle level of triglyceride percentile (HTG Mid).*

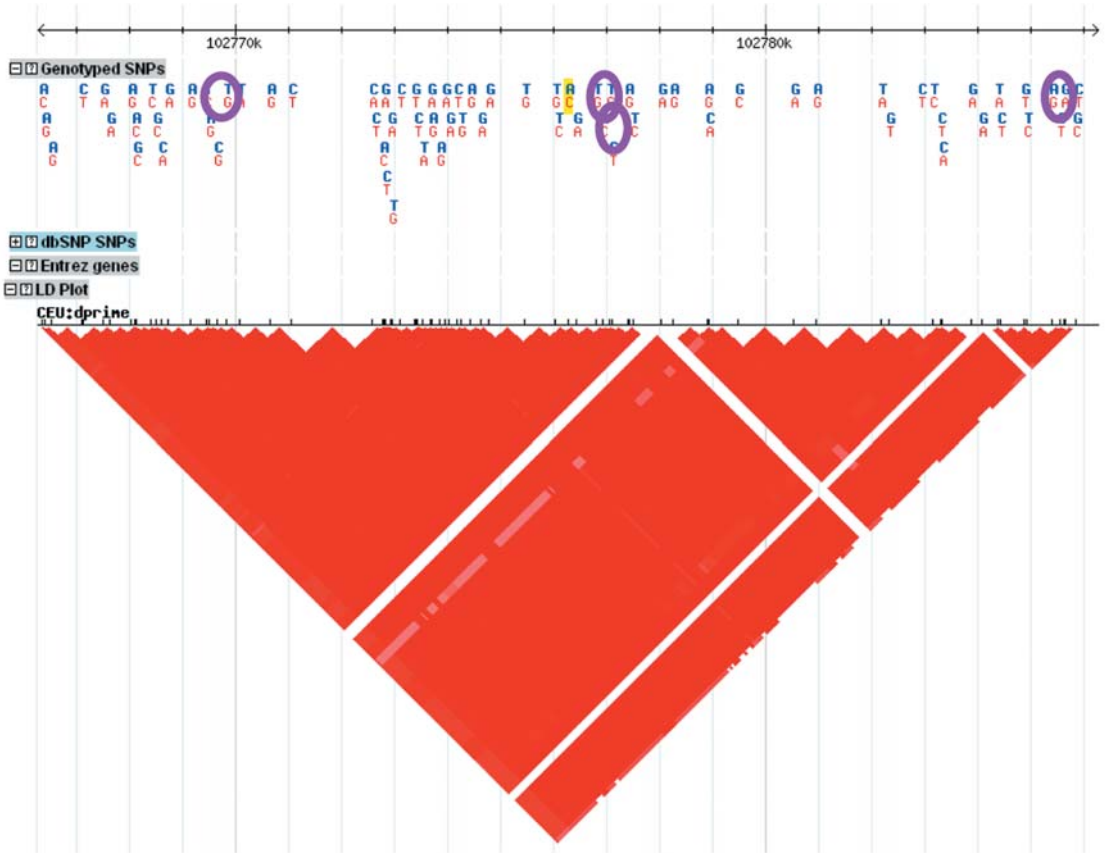

Figure 2. *Regional plot of the associated region at SLC10A2. The significant association at a locus near SLC10A2 was identified using Sequenom iPLEX. The graph is generated from hapmap, and the ruler at the top represents the physical position on Chr13. There are four SNPs (marked by four purple circles) from this region showing significant association with bexarotene-induced hypertriglyceridemia, and they are in strong LD block which is displayed in red triangles or blocks. The LD plot was generated using hapmap website's default settings, the dark red represents d-prime from CEU larger than 1.*

with Affymetrix 500K SNP array (Figure 1B). From the initial genome-wide scan, 255 SNPs were selected for the Stage II genotyping study using all except 3 available clinical samples with Sequenom iPLEX assays. Among the 400 samples, 147 samples (73 cases + 74 controls) were selected as the verification group, and the other 253 samples (76 cases + 48 controls + 129 HTG Mid) were termed the replication group (Figure 1B). To perform association analysis, *p*-values were first calculated based on the allelic frequency differences between cases and controls (Table II). Genotypic tests were also performed using triglyceride percentile as the quantitative trait in the three groups (Table III). For this analysis, individuals with the middle level of triglyceride percentile were included in the replication group. Significant associations were declared for SNPs with *p*-values ≤0.05. By combining these two approaches and criteria for significance, 14 SNPs showing significant associations with triglyceride level were found (Tables II and III).

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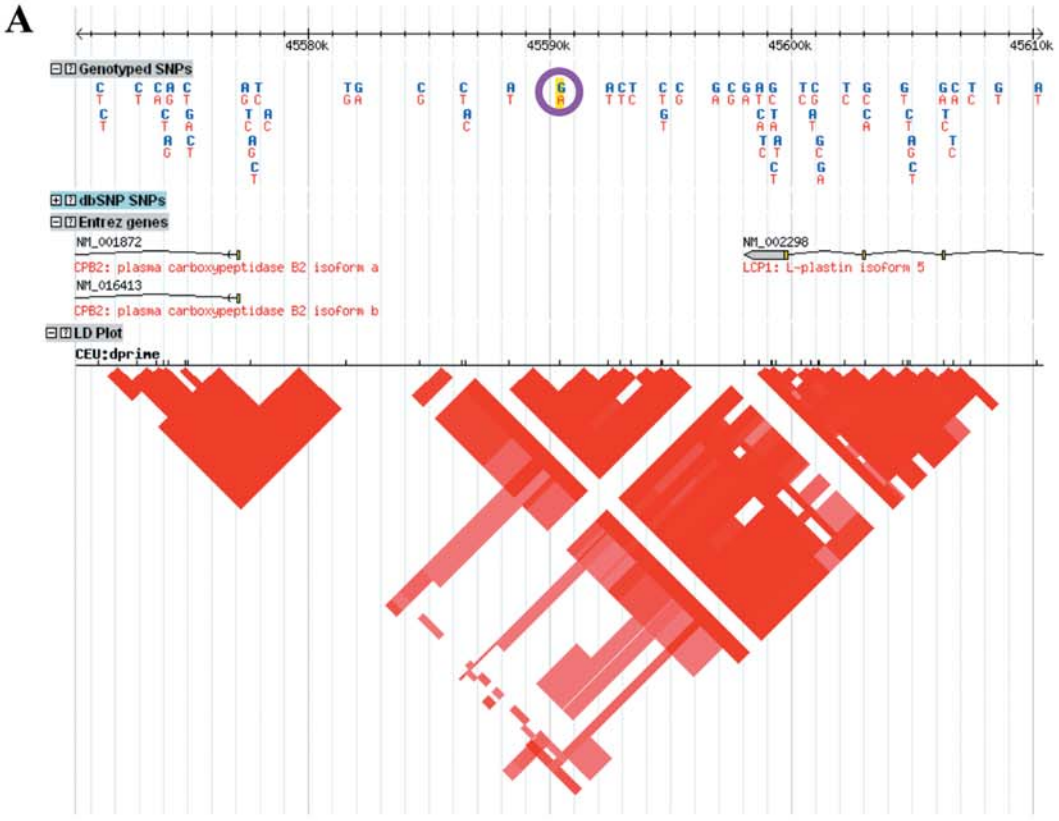
Figure 3. *Associated and functional analysis of LCP1. A: Physical location of rs7334509 on Chr13 and its associated genes. The number along the ruler at the top indicates the physical position on Chr13, and SNPs in this region are shown in two-letter code corresponding to the two alleles of each SNP. rs7334509 is highlighted by a purple circle. The two known genes located in this region, CPB2 and LCP1, are also displayed. The introns of the genes are shown by black curved lines, and exons are illustrated by a box, with coding region shown in yellow and untranslated regions shown in grey. The LD plot was generated using hapmap website's default settings, the dark red represents d-prime from CEU larger than 1. B: Regulation of LCP1 expression in MDA-MB-231 (MDA) cells and its derivative paclitaxel-resistant MDA-PR cells. LCP1 expression data was obtained from the microarray experiment using four groups of samples: i) MDA parental cells; ii) MDA derived cells resistant to paclitaxel (MDA-PR); iii) MDA-PR cells treated with 30 nM paclitaxel (3 days on, 7 days off) for 3 months; iv) MDA-PR cells treated with 30 nM paclitaxel/1 μM bexarotene (3 days both, 7 days only bexarotene) for 3 months. Each group had four replicates and different groups are shown in a different shade of blue color. The LCP1 expression signal of each sample is labeled at the end of the bar graph. Comparing to MDA parental cells, LCP1 expression in MDA-PR is 4.3-fold lower with p-value=1.50×10^{-7}. LCP1 expression was significantly up-regulated by 3.8-fold in MDA-PR cells treated with paclitaxel and bexarotene compared to MDA-PR cells (p-value=6.86×10^{-6}).*


A
45580k
45590k
45600k
45610k
Genotyped SNPs
dbSNP SNPs
Entrez genes
NM_001872
CPB2: plasma carboxypeptidase B2 isoform a
NM_016413
CPB2: plasma carboxypeptidase B2 isoform b
NM_002298
LCP1: L-plastin isoform 5
LD Plot
CEU:dprime

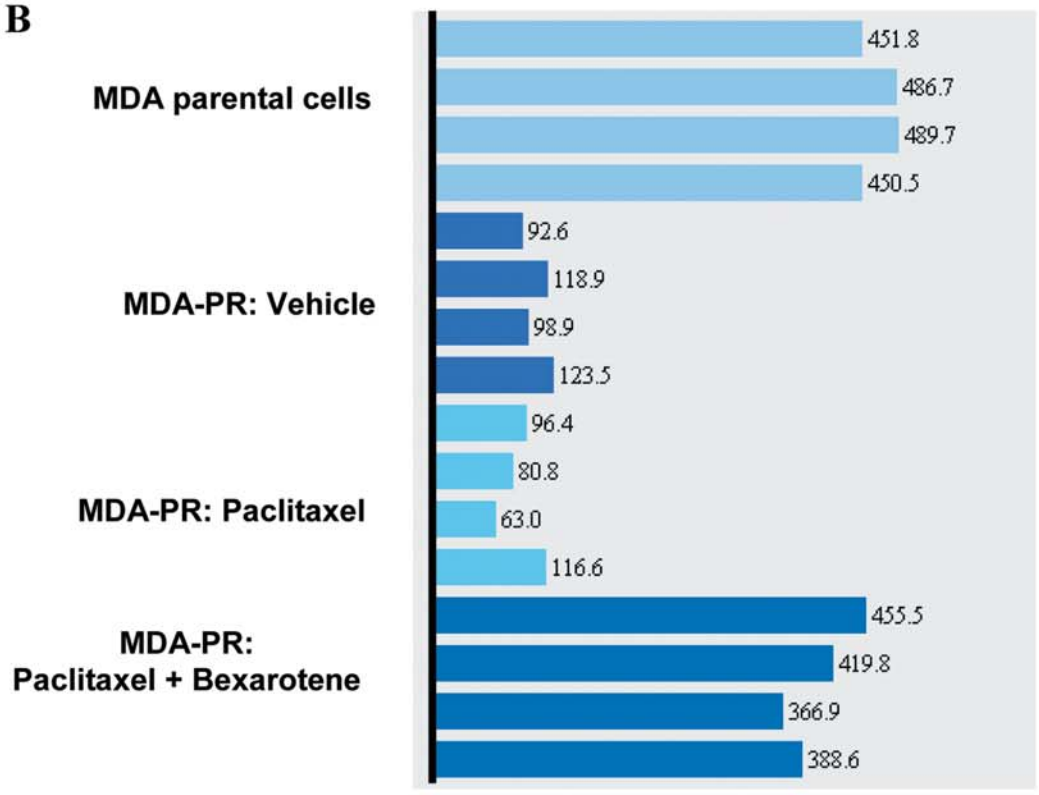

B
MDA parental cells
MDA-PR: Vehicle
MDA-PR: Paclitaxel
MDA-PR:
Paclitaxel + Bexarotene
451.8
486.7
489.7
450.5
92.6
118.9
98.9
123.5
96.4
80.8
63.0
116.6
455.5
419.8
366.9
388.6

Table II. *Association of SNPs with hypertriglyceridemia in case-control analysis. Associations were calculated with two-allele model in the case-control test. The p-values which were lower than 0.05 are highlighted in bold, and the associated SNPs whose p-values are lower than 0.05 in all three tests are with asterisks. Minor allele frequency (MAF) for case and control are listed under the corresponding columns.*

		Verification group (n=141)			Replication group (n=118)			Combined group (n=259)		
SNP	Gene name	Case MAF	Control MAF	*P*-value	Case MAF	Control MAF	*P*-value	Case MAF	Control MAF	*P*-value
rs7434820	*FLJ46481*	0.432	0.219	**2.51E-04**	0.393	0.284	0.1006	0.413	0.245	**1.22E-04**
rs4572960*	*ITGA1/PELO*	0.348	0.645	**1.53E-06**	0.382	0.537	**0.0263**	0.369	0.609	**1.59E-07**
rs10058324*	*ITGA1/PELO*	0.297	0.574	**1.60E-04**	0.373	0.535	**0.0197**	0.335	0.552	**1.59E-05**
rs1051853*	*SPOCK1*	0.045	0.189	**1.44E-03**	0.059	0.143	**0.0450**	0.052	0.165	**2.53E-04**
rs6997581*	*CSMD1/MCPH1*	0.250	0.600	**5.71E-09**	0.382	0.556	**0.0104**	0.326	0.609	**3.16E-10**
rs2631686*	*PCGF5*	0.272	0.500	**9.24E-05**	0.400	0.543	**0.0319**	0.337	0.517	**3.80E-05**
rs1795505*	*LIN7A*	0.117	0.229	**1.66E-02**	0.123	0.238	**0.0259**	0.120	0.232	**1.07E-03**
rs1184776*	*LIN7A*	0.078	0.214	**1.78E-03**	0.101	0.214	**0.0203**	0.090	0.214	**1.12E-04**
rs7334509*	*CPB2*	0.490	0.346	**3.50E-02**	0.592	0.372	**0.0037**	0.540	0.357	**3.35E-04**
rs6491738	*SLC10A2*	0.234	0.455	**2.11E-04**	0.291	0.386	0.1392	0.264	0.427	**1.64E-04**
rs7333033*	*SLC10A2*	0.261	0.458	**5.61E-04**	0.268	0.404	**0.0279**	0.264	0.437	**3.72E-05**
rs7338381	*SLC10A2*	0.254	0.444	**7.91E-04**	0.268	0.383	0.0615	0.261	0.420	**1.26E-04**
rs3916931	*SLC10A2*	0.225	0.431	**2.36E-04**	0.261	0.362	0.0972	0.243	0.403	**9.09E-05**
rs1506011*	*Unknown*	0.589	0.246	**2.33E-08**	0.393	0.238	**0.0213**	0.492	0.243	**3.88E-08**

Table III. *Association of SNPs with hypertriglyceridemia using quantitative trait analysis. The p-values which are lower than 0.05 are highlighted in bold, and the associated SNPs that met this threshold in all three tests are indicated with an asterisk. The two alleles are represented by A and B, thus homozygous are AA and BB, and heterozygous are represented by AB. Average of triglyceride levels in patients carrying the indicated genotype are listed under "Mean TRIG" column. §NA: No mean available because there is only one sample in this genotype.*

				Verification group (n=141)		Replication group (n=244)		Combined group (n=385)	
SNP	Chromosome	Position	Gene name	Mean TRIG AA/AB/BB	*P*-value	Mean TRIG AA/AB/BB	*P*-value	Mean TRIG AA/AB/BB	*P*-value
rs7434820*	4	6081537	*FLJ46481*	41/56/66	**1.21E-03**	46/50/54	**0.0489**	44/53/58	**2.34E-04**
rs4572960*	5	51608341	*ITGA1/PELO*	64/49/30	**1.54E-05**	56/48/47	**0.0245**	59/48/40	**1.52E-06**
rs10058324*	5	51679280	*ITGA1/PELO*	44/58/71	**2.65E-03**	46/47/55	**0.0243**	45/50/61	**1.28E-04**
rs1051853*	5	136341700	*SPOCK1*	NA§/29/62	**3.66E-03**	26/45/50	**0.0182**	36/39/54	**3.42E-04**
rs6997581*	8	5321568	*CSMD1/MCPH1*	63/65/28	**1.18E-07**	52/52/43	**0.0171**	55/57/36	**4.71E-08**
rs2631686	10	93043517	*PCGF5*	28/48/58	**3.00E-04**	43/53/50	0.0722	38/51/54	**1.27E-04**
rs1795505*	12	79771464	*LIN7A*	53/30/40	**3.38E-02**	52/48/41	0.0502	52/41/41	**3.76E-03**
rs1184776*	12	79776533	*LIN7A*	53/33/29	**5.46E-03**	52/43/43	**0.0247**	52/39/39	**3.71E-04**
rs7334509	13	45590412	*CPB2*	43/50/63	6.17E-02	40/52/52	**0.0041**	41/51/55	**1.17E-03**
rs6491738*	13	102769437	*SLC10A2*	59/41/34	**9.67E-04**	52/50/43	**0.0395**	55/46/40	**1.49E-04**
rs7333033*	13	102776263	*SLC10A2*	59/41/37	**1.54E-03**	52/50/40	**0.0077**	55/47/39	**3.18E-05**
rs7338381*	13	102776418	*SLC10A2*	58/41/38	**3.79E-03**	52/51/40	**0.0141**	54/47/39	**1.38E-04**
rs3916931*	13	102784358	*SLC10A2*	59/42/33	**7.04E-04**	53/48/43	**0.0221**	55/46/40	**6.15E-05**
rs1506011	21	23490212	*Unknown*	32/50/75	**1.47E-07**	48/48/57	0.1491	43/49/66	**1.28E-06**

Associated loci. The 14 SNPs with significant associations with hypertriglyceridemia (Tables II and III) are located on 9 loci. Four significantly associated SNPs are located within the same linkage disequilibrium (LD) block on chromosome 13 (Figure 2). This region lies approximately 200 kb upstream of *SLC10A2*, a gene responsible for reabsorption of bile acids from the small intestine. The second region with multiple SNPs showing a strong association is located on chromosome 12, where rs1795505 and rs1184776 are only about 5000 nucleotides apart. Both SNPs lie in the intron region of Lin-7 homolog A (*Lin7A*), but this region is in an LD block containing the 2nd and 3rd exons of the *Lin7A* gene.

Another associated SNP, rs7334509, lies between *LCP1* and carboxypeptidase B2 (*CBP2*), and resides in an LD block containing the 3' half of the *LCP1* (Figure 3A). Bexarotene has been shown to resensitize breast cancer cells which had become chemo resistant after prolonged treatment with chemotherapy agents, such as paclitaxel (5). Examination of gene expression data from the microarray study with MDA-PR cells resistant to paclitaxel revealed that *LCP1*, which lies close to rs7334509, was up-regulated by co-treatment with bexarotene (Figure 3B). In the microarray study, expression of *LCP1* was significantly reduced in the paclitaxel resistant MDA-PR cell line compared to its parent cell line. In MDA-PR cells treated with bexarotene and paclitaxel, LCP1 expression was significantly up-regulated, to about the same level as that in parental MDA-MB-231 cells (Figure 3B). However, treatment with paclitaxel alone had no effect on the expression of *LCP1*.

For the rest of the significant SNPs, three have *p*-values $\leq 5\times10^{-7}$ (rs6997581, $p=3.16\times10^{-10}$; rs1506011, $p=3.88\times10^{-8}$; rs4572920, $p=1.59\times10^{-7}$) (Table II,). The most strongly associated SNP, rs6997581, is distantly surrounded by two genes, 400 kb to *CSMD1* (CUB and Sushi multiple domains 1) and 900 kb to *MCPH1* (microcephaly, primary autosomal recessive 1). rs4572960 and the closely located rs10058324 (1.59×10^{-5}) are about 70 kb from each other and in weak LD, with D'=0.8. There are two genes, *PELO* (Pelota homolog) and *ITGA1* (integrin alpha 1), which are located 500 kb away from this locus. *PELO* encodes a protein which contains a conserved nuclear localization signal, and *ITGA1* encodes the alpha 1 subunit of integrin receptors. No known gene was found to reside in the vicinity of rs1506011. The remaining three significant SNPs, rs7434820 (*FLJ46481*), rs1051853 (sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1 (*SPOCK1*)), and rs2631686 (polycomb group ring finger 5 (*PCGF5*)) exhibit modest association with hypertriglyceridemia.

Discussion

RXR is able to form heterodimers with a number of nuclear receptors, such as retinoid acid receptors, peroxisome proliferator-activated receptors (*PPAR*), Liver X receptors, and farnesoid X receptor, and is a pleiotropic regulator involved in many biological pathways. With the development of RXR-specific agonists, such as bexarotene, tremendous potential exists to benefit many critical therapeutic areas, including metabolic syndrome, dermatologic disease, treatment of tumors and cancer prevention (13, 14). Clinical applications of retinoids in general, and RXR agonists as well, have been in part limited by associated undesirable side-effects, *e.g.*, the elevation of serum triglycerides. Although the induction of hypertriglyceridemia by RXR agonists has been known for a long time, the molecular mechanism underlying this phenomenon remains elusive. One of the associated loci identified from this study, *SLC10A2*, is an attractive candidate that might be involved in rexinoid-induced hypertriglyceridemia. *SLC10A2* is a gene responsible for reabsorption of bile acids from the small intestine. Bile acids are known to affect serum triglyceride levels (15-18), and as the major component for bile acid uptake, the activity of *SLC10A2* is closely related to triglyceride levels (19, 20). Moreover, *SLC10A2* is known to be regulated by RXR at the transcriptional level, probably through forming a heterodimer with *PPARα*, a nuclear receptor known to participate in lipid metabolism (21, 22). Down-stream conversion of triglycerides into fatty acids also generates signaling molecules that activate a number of nuclear receptors, many of which form hetero-dimer partners with the RXR receptors, potentially triggering other cellular mechanisms controlling cancer cell growth.

This study also allows insight into antitumor activity of the bexarotene due to the correlation between survival and highly elevated triglyceride levels. One associated locus, rs7334509, is located near *LCP1*, which is also called L-Plastin. Plastins are a family of actin-binding proteins that are conserved throughout eukaryote evolution and expressed in most tissues of higher eukaryotes. These proteins share the unique property of cross-linking actin filaments into tight bundles, and they are primarily involved in regulation of the actin cytoskeleton. L-Plastin is predominantly expressed in hematopoietic cells, but has also been shown to be expressed in most human cancer cells and might be involved in DNA repair, tumor cell migration and invasion (23, 24). In our microarray study, expression of *LCP1* in the paclitaxel-resistant MDA-PR cell line was significantly up-regulated by a combination of bexarotene and paclitaxel treatment. This result implies that *LCP1* might be involved in the antitumor action exerted by bexarotene. Interestingly, the reversal of *LCP1* expression mediated by bexarotene is unique to paclitaxel-resistant tumor cells because it was not observed in breast cancer cells resistant to either cisplatin or doxorubicin, which target DNA instead of tubulin (data not shown). Recent studies have found both actin and tubulin to be involved in tumor cell-resistance to chemotherapy (25-27). Thus, the *LCP1* polymorphism identified in this study may causally or functionally influence individual responses to bexarotene as an antitumor agent, and this hypothesis needs to be tested in other tumor cell lines including lung cancer cells.

Using DNA extracted from a suboptimal source such as plasma, the genotype call rates in our study are lower than the typical call rates for other genotyping studies where DNA was extracted from optimal DNA sources such as whole blood. Detailed analysis of the genotyping raw data generated from iPLEX assays showed that many of the no calls were due to very low or no signal from either allele, but

reliable genotype calls still could be made when signals are substantially higher than background noise (data not shown). Studies from Lu *et al*. and Park *et al*. on whole genome amplification samples also showed that high concordance genotype calls could be obtained from samples with low overall call rates (28, 29). In addition, multiple SNPs were genotyped in the same LD block, since it is unlikely that a false positive result would continually arise in subsequent genotyping and analyses. For instance, the four significant SNPs identified in the LD block close to *SLC10A2* are unlikely to have been driven by multiple genotyping errors that happen to go in the direction of an association. In addition, some of the genes related to these associated SNPs are related to the functions of bexarotene.

Plasma samples were not collected from patients in the chemotherapy-only control groups. Thus, we can not definitely distinguish the associated polymorphisms identified in this study as predictor for bexarotene action from prognostic markers for patients who might survive longer regardless of bexarotene treatment status. We also recognize that unplanned retrospective analysis of clinical trials might lead to statistical artifacts. However, the correlation between survival and triglyceride level induced by bexarotene was observed in two very large, randomized, independent phase III trials (9, 10) which evaluated different chemo therapy agents with or without bexarotene across nearly 250 clinical sites worldwide. The patient populations were very uniform due to the strict inclusion criteria, and all patients had advanced stage IIIb or stage IV NSCLC qualifying for first-line treatment. Moreover, similar correlations between high triglyceride levels and bexarotene efficacy were also revealed by retrospective analysis in other bexarotene phase II lung cancer and renal cell carcinoma trials, as well as trials in cutaneous T cell lymphoma (11).

Overall, the associated SNPs identified from this study provide a new hypothesis for rexinoid-induced hypertriglyceridemia and its antitumor action. These intriguing results warrant confirmation and further exploration, which might eventually lead to development of patient selection criteria to maximize the efficacy of RXR modulators to treat important diseases such as metabolic disorders and cancer. These polymorphisms are also promising candidates for use as genomic markers to predict bexarotene response, and their clinical utility could be further investigated in prospective clinical trials.

Competing Interest Disclosure

Wen Luo, Keith B. Marschke, Shi-Chung Ng, Thomas W. Hermann, J.K. Zhang, Jennifer Sanders, Patricia Tooker, Zofia E. Dziewanowska, Andres Negro-Vilar, and Martin D. Meglasson are or were employed by Ligand Pharmaceuticals, who discovered and developed bexarotene. Nicholas J. Schork was a consultant of Ligand Pharmaceuticals.

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