Leptin and leptin receptor genotypes and colon cancer: Gene–gene and gene–lifestyle interactions

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Abstract

Leptin may play an important role in colorectal cancer because of its role in energy balance, insulin and inflammation. We evaluated the LEP rs2167270 (19 G > A) and rs7799039 (−2548 G > A) polymorphisms and the leptin receptor, LEPR rs6588147 (located in intron 2), polymorphism with risk of developing colon cancer in a study of 1,567 cases and 1,965 controls. We evaluated the effects of the polymorphisms with body mass index (BMI), recent use of aspirin/NSAIDs and genetic variations in genes related to insulin signaling pathways including insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3), and insulin- related substrates 1 and 2 (IRS1, IRS2) and the vitamin D receptor (VDR). We observed a slight reduction in colon cancer risk with the AA LEP rs2167270 genotype (OR 0.79 95% CI 0.64, 0.98) and although not reaching statistical significance, with the combined GG LEP rs2167270 and GG LEPR rs6588147 (OR 0.70, 95% CI 0.49, 1.02) genotypes. BMI did not interact with any of these polymorphisms to alter colon cancer risk. However, recent aspirin/NSAID use significantly interacted with both LEP polymorphisms. Likewise, variants of IGF1 and IRS2 interacted with the LEP rs2167270 polymorphism. VDR polymorphisms interacted with all LEP and LEPR polymorphisms. These data support an association between LEP and colon cancer. They also suggest that the mechanisms linking leptin to colon cancer may be independent of energy balance.

Keywords

colon cancer; leptin; leptin receptor; polymorphisms; VDR; aspirin; obesity; insulin; inflammation

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Leptin is a 16 kDa glycolprotein product of the leptin gene (*LEP*), which is expressed almost exclusively (>95%) by adipocytes. [1] Initial interest in leptin focused on its role in obesity but it is rapidly becoming evident that its physiological properties extend beyond those associated with energy homeostasis. In addition to regulation of energy balance, leptin, has been associated with reproductive factors, inflammatory response, insulin signaling, bone remodeling and neuroendocrine function. [2] The leptin receptor, a class I pro-inflammatory cytokine, is a member of the cytokine receptor family that includes IL-6. The leptin receptor plays a key role in how leptin functions. When leptin binds to the leptin receptor, Janus kinase (JAK2), a tyrosine kinase is activated to initiate downstream signaling of suppressors of cytokine signaling 1 and 3 (SOCS) and signal transducer and activator of transcription-3 (STAT).[2–4] Of particular importance for cancer is the influence of leptin on SOCS which in turn limits insulin signaling; SOCS3 has been shown to limit leptin signaling. Studies have

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shown that leptin also can stimulate expression of the inflammatory marker CRP (C-reactive protein).[5]

There is growing evidence that nuclear hormone receptors regulate the action of leptin and other class I cytokines.[6] However, data also suggest that hormone receptors, such as the vitamin D receptor, might play an important role in regulating leptin; vitamin D_3 has been shown to inhibit leptin secretion in human adipose tissue.[7] Leptin has been shown to attenuate expression of renal 25-hydroxyvitamin D(3)-1α-hydroxylase in mice via the leptin receptor.[8]

Although data directly linking leptin to colon cancer are limited, some studies have shown increased risk of colon and colorectal cancer with high serum leptin levels.[9–11] Observations that the leptin receptor is expressed in human colon cancer cells lines and CRC derived human tissue lends additional support to leptin playing a role in CRC.[12] Energy balance, adiposity, insulin, inflammation and vitamin D have been associated with colon cancer and also are associated with leptin and its receptor.[13–17]

Polymorphisms in the leptin and leptin receptor gene have been studied in conjunction with leptin levels and obesity and might also provide insight into associations with cancer. In one study, having an A allele at the −2548 site (rs7799039) in the leptin gene (*LEP*) was associated with higher expression of the leptin receptor as well as with prostate cancer.[18–20] Lower leptin binding to the leptin receptor has been shown with the A allele of the Gln223Arg SNP (rs1137101) of the leptin receptor (*LEPR*) gene.[21] Two other SNPs, Ser343Ser (rs790419) in exon 1 and Lys109arg (rs1137100) in exon 4 of the *LEPR* gene, have been associated with obesity in women in France.[22]

To obtain a better understanding of the association between leptin and colon cancer, we evaluated 2 variants of the *LEP* gene, the more commonly studied rs7799039 as well as rs2167270 and 1 variant of the *LEPR* gene, rs6588147 with risk of developing colon cancer. These variants were selected because of previous reports of their reported functional importance[23] and their being in linkage disequilibrium with other common LEP markers such as rs2167270 and rs3828942. These analyses, therefore, serve as an initial evaluation of the potential importance of *LEP* and *LEPR* genes in colon cancer. In addition to evaluating the independent associations between *LEP* and *LEPR* polymorphisms and colon cancer, we evaluated the joint effects of *LEP* and *LEPR* polymorphisms with factors available in our dataset for which we believe there is a biological basis for an interaction. We evaluated how *LEP* and *LEPR* polymorphisms were related to BMI, an indicator of energy balance since leptin has been associated with energy homeostasis. We evaluated recent use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), possible indicators of inflammation, since LEP and LEPR are pro-inflammatory cytokines. We evaluated genes involved in an insulin pathway, including insulin-like growth factor 1 (*IGF1*), insulin-like growth factor binding protein (IGFBP3), insulin-receptor substrates 1 and 2 (*IRS1* and *IRS2*) since leptin levels are associated with insulin. We evaluated the vitamin D receptor (VDR) genotypes given reports of the associations between the nonsteroid nuclear receptor VDR and leptin levels.

Methods

Data for the study come from a case-control study of first primary colon cancer (ICD-O 2nd edition codes 18.0, 18.2 to 18.9) diagnosed between October 1, 1991 and September 30, 1994 conducted in the Northern California Kaiser Permanent Medical Care Program (KPMCP), the Wasatch Front area of Utah and the Twin Cities Metropolitan area of Minnesota. The study was approved by the University of Utah Institutional Review Board as well as Institutional Review Boards at the Kaiser Permanente Medical Care Program of Northern California (KPMCP) and the University of Minnesota. Case eligibility was determined by the Surveillance

Epidemiology and End Results[24] Cancer Registries in Northern California and in Utah and the Minnesota Cancer Surveillance System. Eligibility included being between 30 and 79 years of age at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer[14] and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis or Crohn's disease. Of cases contacted, 83% participated at KPMCP, 76% in Utah and 67% in Minnesota.

Controls were frequency matched to cases by sex and by 5-year age groups. At the KPMCP, controls were randomly selected from membership lists. In Utah, controls 65 years and older were randomly selected from lists provided by the Centers for Medicare and Medicaid Services (formerly HCFA) and controls younger than 65 were randomly selected from driver's license lists. In Minnesota, controls were randomly selected from driver's license lists. Of controls contacted for the colon cancer study, 73% participated at KPMCP, 53% participated from Minnesota and 69% participated from Utah.

Data collection

Trained and certified interviewers collected diet and lifestyle data.[25,26] The referent year for the study was the calendar year ~2 years before date of diagnosis (cases) or selection (controls). Information was collected on demographic factors such as age, sex and study center; diet, physical activity, aspirin and nonsteroidal drug use, body size, and other lifestyle factors including medical, family and reproductive history.

Genotyping

DNA was extracted from blood drawn from study participants. TaqMan assays for Leptin markers *LEP* rs2167270 (19 G > A) and *LEP* rs7799039 (~2548 G > A), and Leptin Receptor marker *LEPR* rs6588147 (located in intron 2) were purchased from Applied Biosystems (Foster City, CA). Genotyping was performed on 20 ng of genomic DNA as described by the manufacturer. Data were collected as .fluorescent endpoint measurements of the TaqMan reactions using a 7900HT sequence detection instrument. Control samples representing all 3 possible genotypes were included at 4 positions each in every 384-well tray. In addition, internal replicates representing >1% of the sample set were blinded and included.

The intron 8 *Bsm* I (rs154410) and Fok1 (rs10735810) *VDR* polymorphisms were amplified from genomic DNA and digested as described previously.[27,28] Presence of the restriction site was scored as allele "b," and absence of the restriction site was scored as allele "B." Presence of the restriction site was scored as allele "f," absence of the restriction site was scored as allele "F." Genotyping of *IGF1*, IGFBP3, *IRS1*, and *IRS2* have been described.[29,30] The *IGF1* CA repeat, the *IGFBP3* −202 A>C, the *IRS1* G972R and the *IRS2* G1057D polymorphisms were evaluated.

Statistical methods

SAS statistical package version 9.1 was used to conduct the analyses. We evaluated the distribution of the *LEP* and *LEPR* genotypes and the independent associations of these genetic polymorphisms with colon cancer. Odds ratios (OR) and 95% confidence intervals (CI) are used to report associations obtained from multiple logistic regression models. Associations were evaluated for men and women separately, for proximal and distal colon tumors and by age. Associations were adjusted for age, sex, center and race because these variables were matching variables in the study design. Since over 90% of study participants were non-Hispanic white, it was not feasible to assess associations for different racial and ethnic groups. Factors such as body mass index,[31,32] physical activity and family history of colorectal cancer, cigarette smoking and dietary composition did not alter associations and therefore are not included as covariates in the final logistic models. We report the joint effect of *LEP* and

LEPR genotypes and BMI (kg/m^2), aspirin/NSAIDs, Bsm1 and Fok1 *VDR* polymorphisms, and *IGF1, IGFBP3, IRS1* and *IRS2* polymorphisms. For the *IRS1, IRS* and Fok1 *VDR* genes, we evaluated a dominant model because of similar associations across heterozygote and homozygote variant genotypes. For joint effects, multivariate logistic regression models were used to calculate odds ratios for each category of exposure and each genotype. Because we did not observe significant differences in effect by gender for interactions, we present interaction models for the entire population. Effect modification between genotypes and exposure variables were evaluated by likelihood ratio test for a multiplicative-interaction term in the logistic regression model.

Results

The AA genotype of the *LEP* rs2167270 SNP was associated with reduced risk of colon cancer (OR 0.79 95% CI 0.64, 0.98) (Table I). The rs7799039 *LEP* SNP was not significantly associated with risk of colon cancer among men, women or everyone combined. Among men, the *LEPR* rs6588147 SNP reduced risk of colon cancer (OR 0.71 95% CI 0.52, 0.97). Evaluation of both the *LEPR* and *LEP* SNPs together, showed the greatest reduction in colon cancer risk for the AA genotype of the *LEP* rs2167270 SNP and the GG genotype of the rs6588147 *LEPR* SNP. However, the interactions between *LEPR* and *LEP* were not statistically significant. Subsequent analyses are therefore presented for each SNP separately with men and women combined.

Because of previous associations with BMI observed only for men and for women who were recently exposed to estrogen because they were premenopausal or taking hormone replacement therapy,[15] we present analyses for that group only. We did not observe a statistically significant interaction between BMI level and any of the *LEP* and *LEPR* polymorphisms (Table II). However, the GG genotype of the *LEPR* gene appeared to halve the risk of colon cancer for those with normal BMIs and those with a BMI of 30 or more. Among those with normal weight, the AA genotype of the *LEP* rs2167270 polymorphisms was associated with the greatest reduction in the risk of colon cancer (OR 0.63, 95% CI 0.39, 1.03) with a BMI of <25. There were no statistically significant interactions between these polymorphisms and other components of energy balance such as physical activity and energy intake (data not shown).

We observed a statistically significant interaction between use of aspirin/NSAIDs during the past 2 years and both *LEP* polymorphisms and risk of colon cancer (Table III). For both the *LEPR* and *LEP* rs2167270 markers, those with the lowest colon cancer risk were individuals who currently used aspirin/NSAIDs and had the GG (*LEPR*) and AA (*LEP*) genotypes. For the rs7799039 *LEP* polymorphism, those who used aspirin and had the GG genotype had the lowest colon cancer risk. The association with leptin genotypes and nonusers of NSAID is null.

Evaluation of insulin-related genes and risk of colon cancer showed significant interactions between both *IGF1* and *IRS2* polymorphisms and the *LEP* rs2167270 polymorphism (Table IV). People with the AA genotype of the *LEP* rs2167270 polymorphism and did not have a 19 CA repeat of the *IGF1* gene were at reduced risk of colon cancer (OR 0.63, 95% CI 0.38, 1.06) compared to people with the 19/19 CA repeat genotype and the AA rs2167270 *LEP* polymorphism (OR 1.02 95% CI 0.72, 1.44). Similar interactions were observed with the *IRS2* G1057D polymorphism, although the greatest reduction in risk was observed for the combination of the GG *IRS2* genotype and the AA *LEP* rs2167270 genotype.

The rs65888147 *LEPR* and the rs7799039 *LEP* polymorphisms significantly interacted with the Bsm1 *VDR* gene (Table V). For the rs67888147 marker, those with a B allele had the greatest reduction in risk if the also had the GG genotype (OR 0.75 95% CI 0.55, 1.02). Those with the *VDR* bb genotype and the AA rs7799039 *LEP* genotype had statistically significantly

lower risk than those with the bb and GG genotypes. Both of the *LEP* markers interacted the Fok1 *VDR* polymorphisms. Among those with the f allele, those with the GG genotype of rs7799039 *LEP* and the AA genotype of the rs2167270 marker had the greatest reduced risk of colon cancer.

Discussion

In this study, we evaluated the associations between the rs6588147 *LEPR* rs7799039 *LEP*, and the rs2167270 *LEP* markers and risk of colon cancer. The two leptin markers were chosen because they represent polymorphic loci that have previously been associated with variability in secreted leptin levels and obesity. [19,20,23] Marker rs6588147 located in intron 2 of the leptin receptor gene was chosen for its proximity to the 5′ end of the gene and its relatively common minor allele frequency of ~0.39 in the Caucasian population. The LEP markers studied also are in linkage disequilibrium with two other LEP markers, rs2167270 and rs3828942. We observed that various combinations of the *LEPR* and *LEP* genotypes may alter colon cancer risk, providing support for a link between leptin and its receptor and colon cancer. Because of the diverse biological activities of leptin, we evaluated several factors for which we believe there was a physiological basis for interaction with *LEP* and *LEPR* and could influence colon cancer risk. Our data provide little support for *LEP* and *LEPR* polymorphisms modulating energy balance, although they do support associations with factors related to insulin and inflammation as well as *VDR*

Leptin is derived from adipocytes and circulating levels of leptin are associated with adipose tissue mass.[33] Leptin influences regulation of food intake and energy expenditure in conjunction with its receptors.[34,35] Studies of leptin, the protein coded by the ob gene in mice, showed that mice with a mutation in the gene developed obesity and subsequent treatment with leptin caused weight loss.[36,37] Therefore, much of the early work on leptin has focused on its associations with obesity. Because of the associations between energy balance as a contributor to the development of several types of cancer, including colon cancer,[14] assessment of leptin as a modulator of colon cancer is reasonable. Data from a cohort study in Norway detected an almost threefold increased risk of colon cancer among people with high leptin levels,[11] the association was independent of BMI, suggesting that mechanisms other than energy balance were involved. Similar associations, although slightly weaker, were observed in a nested case-control study conducted in Sweden.[9] We observed a weak protective effect with *LEP* and *LEPR* variant alleles of the polymorphisms assessed. However, our data suggest that the *LEP* and *LEPR* polymorphisms assessed do not interact with BMI or energy-balance related factors such as physical activity and energy intake. It is possible that other *LEP* and *LEPR* polymorphisms may have different associations with BMI, although it also is possible that *LEP* and *LEPR* associations with colon cancer risk include biological mechanisms other than energy balance. It is also recognized that other composition of body composition, such as total adipose tissue mass and distribution of adipose tissue may be important to clearly define the association between leptin and body size.

Other possible effects of leptin on colon cancer risk might involve inflammation-related pathways. It is now recognized that adipose tissue secretes adipocytokines such as adiponectin and leptin. Leptin and its receptor are considered pro-inflammatory cytokines and play a major role in modulating inflammation and immune response.[33,38] Studies also have shown that prostaglandin E2 stimulates leptin secretion from cultured human adipose-tissue cells and that COX2 inhibitors prevented the increased leptin production. [39] Our results showed a statistically significant interaction between recent use of aspirin/NSAIDs and *LEP* polymorphisms suggest that leptin may influence colon cancer through mechanisms involving COX2 inhibitors and that greater pro-inflammatory response possibly associated with genotype may be modified by aspirin/NSAID.

Insulin and leptin interact at multiple levels within a complex network of adipose tissue signaling pathways,[1] providing another mechanism that could link leptin to colon cancer. It is thought that insulin is involved in the secretion of leptin from the adipocyte, and thus is involved in the leptin signal-transduction pathway. The pathway that connects IRS-1 to the insulin signaling cascade can be modulated by leptin and the leptin receptor.[33] Several studies have examined the associations between leptin and IGF-1 and IGFBP-3.[40–43] We observed a statistically significant interaction between *LEP* polymorphisms and both *IGF1* and *IRS2* genotypes. Those without a 19 CA repeat of the *IGF1* gene had the greatest reduction in risk of colon cancer if they also had the AA genotype of the rs2167270 *LEP* gene. Studies have shown that the 19/19 CA repeat of the *IGF1* gene is associated with lower serum levels of IGF-1, which may imply lower colon cancer risk.[44] We observed that the non-19/19 *IGF1* genotype was associated with the greatest risk, as expected, but only in the presence of the *LEP* AA genotype, and thus, possibly lower leptin levels.[18] For the same rs216720 *LEP* polymorphism, we observed a statistically significant interaction with *IRS2*, with the greatest reduced risk for those with the AA genotype who also had the GG *IRS2* genotype. These *IRS2* polymorphisms (DD genotype) have been associated with obesity and insulin resistance[45] and therefore, the association with *LEP* may indicate interaction with obesity or insulin.

Of interest is our observation of a statistically significant interaction between the *LEP* and *LEPR* polymorphisms and *VDR* genotypes with colon cancer risk. Given previous work has shown that vitamin D_3 can regulate leptin levels and that leptin can influence expression of renal 25-hydroxyvitamin D_3-1α-hydroxylase in mice there appears to be support for our observed association between *VDR* and *LEP* and *LEPR*.[7,8] Vitamin D, like leptin, is thought to have multiple biological roles that could relate to cancer, including both inflammation and insulin[46] and has been shown to be associated with colon cancer.[47]

There are limitations to our work, including the limited number of polymorphisms examined. The *LEP* polymorphisms we examined were thought to have functional role based on the literature [19,20,23]; however, other polymorphisms may help further define the relationship with colon cancer and the pathways being examined. Even with the limited number of polymorphisms, we have made many comparisons and it is possible that findings are from chance. Thus, it is important that others replicate these observations as a means of validation of our results. Additionally, exposure information was based on participant recall which is subject to bias in any epidemiological study. From previous analyses we know that nonparticipants were more likely to be older and have more advanced disease stage[48]; however, we do not believe that these factors would contribute to associations since we did not observe an association between these genotypes and survival, and associations did not differ by age at diagnosis.

Our results add to the limited available data on the association between leptin and the leptin receptor as they relate to risk of colon cancer. Although we did not observe meaningful interaction between these markers of *LEP* and *LEPR* and indicators of energy balance, we did observe important interactions with indicators of insulin and inflammation-related pathways and with *VDR*. One of the challenges in defining an association between colon cancer, obesity, insulin, inflammation, and leptin is to disentangle their roles and influences upon each other, especially within the context of epidemiologic studies. It remains to be determined whether leptin plays a causal role in the risk of developing CRC, or whether it is merely a marker for other risk-contributing processes. Research to understand the associations and the functionality between leptin and CRC are therefore needed. It is possible that leptin may be at an important junction of pathways that are central to the development of CRC.

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TABLE I

Association between Leptin (*LEP*) and Leptin Receptor (*LEPR*) Genotypes and Colon Cancer

| | | | | | *LEPR* (rs6588147) | | | | | | | | | | | | |
| | | Controls (*n*) | Cases (*n*) | OR[1] | 95% CI | | AA | | | | AG | | | | GG | | |
						Controls (*n*)	Cases (*n*)	OR	95% CI	Controls (*n*)	Cases (*n*)	OR	95% CI	Controls (*n*)	Cases (*n*)	OR	95% CI
Men						477	417	1.00	Reference	454	393	1.00	0.83, 1.21	123	75	0.71	0.52, 0.97
										Combined rs6588147 and rs7799039							
LEP rs7799039	GG	363	287	1.00	Reference	166	146	1.00	Reference	156	118	0.89	0.64, 1.24	37	22	0.70	0.39, 1.25
	GA	487	444	1.19	0.97, 1.46	216	204	1.12	0.83, 1.51	211	200	1.13	0.84, 1.53	58	37	0.76	0.48, 1.23
	AA	210	157	0.98	0.75, 1.27	95	67	0.84	0.57, 1.25	87	74	1.02	0.69, 1.50	28	16	0.68	0.35, 1.32
										Combined rs6588147 and rs2167270							
LEP rs2167270	GG	438	349	1.00	Reference	207	160	1.00	Reference	177	162	1.20	0.89, 1.62	54	27	0.66	0.40, 1.10
	GA	452	424	1.18	0.97, 1.43	190	210	1.44	1.08, 1.91	212	174	1.08	0.81, 1.44	48	39	1.07	0.67, 1.71
	AA	165	111	0.84	0.64, 1.11	79	47	0.76	0.50, 1.16	65	54	1.09	0.72, 1.65	21	9	0.56	0.25, 1.26
Women						411	349	1.00	Reference	396	262	0.80	0.64, 0.99	108	80	0.90	0.65, 1.25
										Combined rs6588147 and rs7799039							
LEP rs2167270	GG	275	219	1.00	Reference	136	117	1.00	Reference	109	77	0.86	0.59, 1.27	28	24	1.09	0.59, 1.99
	GA	459	345	1.01	0.80, 1.27	183	167	1.10	0.79, 1.53	204	134	0.84	0.60, 1.18	57	42	0.95	0.59, 1.52
	AA	188	129	0.92	0.69, 1.24	81	64	1.01	0.66, 1.53	83	51	0.79	0.51, 1.22	23	14	0.78	0.38, 1.59
										Combined rs6588147 and rs2167270							
LEP rs2167270	GG	362	264	1.00	Reference	159	139	1.00	Reference	158	98	0.73	0.52, 1.02	44	27	0.73	0.43, 1.25
	GA	417	345	1.14	0.92, 1.42	185	169	1.06	0.78, 1.44	178	134	0.89	0.65, 1.23	51	40	0.93	0.58, 1.50
	AA	139	84	0.82	0.60, 1.13	66	39	0.67	0.42, 1.06	59	30	0.59	0.36, 0.97	13	13	1.20	0.54, 2.67
Everyone						881	759	1.00	Reference	848	650	0.91	0.79, 1.05	230	155	0.81	0.64, 1.02
										Combined rs6588147 and rs7799039							
LEP rs7799039	GG	634	499	1.00	Reference	301	260	1.00	Reference	265	193	0.88	0.69, 1.14	65	46	0.88	0.58, 1.34
	GA	938	782	1.13	0.97, 1.33	408	368	1.13	0.90, 1.42	414	332	1.03	0.82, 1.29	114	79	0.89	0.63, 1.25
	AA	393	284	0.98	0.80, 1.19	172	130	0.95	0.71, 1.28	169	124	0.92	0.68, 1.23	51	30	0.75	0.46, 1.22
										Combined rs6588147 and rs2167270							
LEP rs2167270	GG	794	611	1.00	Reference	361	297	1.00	Reference	335	260	0.96	0.76, 1.20	97	54	0.70	0.49, 1.02
	GA	867	766	1.17	1.01, 1.35	375	378	1.24	1.00, 1.53	369	307	1.00	0.80, 1.24	99	79	1.02	0.72, 1.42
	AA	304	190	0.79	0.64, 0.98	145	84	0.69	0.50, 0.94	124	83	0.81	0.59, 1.12	34	22	0.79	0.45, 1.39

[1] Adjusted for 5-year age group and race; everyone also adjusted for sex.

TABLE II

Association between BMI and Leptin (*LEP*) and Leptin Receptor (*LEPR*) Gene Polymorphisms and Colon Cancer Risk

| | BMI | | | | | | | | | | | |
| | Normal (<25 kg/m²) | | | | Overweight (25 to <30 kg/m²) | | | | Obese (≥30 kg/m²) | | | |
	Controls (*n*)	Cases (*n*)	OR[1]	95%CI	Controls (*n*)	Cases (*n*)	OR	95%CI	Controls (*n*)	Cases (*n*)	OR	95%CI
Men and women recently exposed to estrogen[2]												
LEPR (rs6588147)												
AA	241	161	1.00		277	215	1.21	0.92, 1.59	105	148	2.04	1.47, 2.83
AG	220	143	1.01	0.75, 1.35	282	210	1.17	0.89, 1.53	115	134	1.78	1.29, 2.45
GG	71	24	0.51	0.31, 0.85	65	47	1.16	0.76, 1.79	39	25	0.95	0.55, 1.65
p interaction	0.25											
LEP (rs7799039)												
GG	155	97	1.00		223	149	1.06	0.76, 1.48	85	103	1.80	1.22, 2.67
GA	279	167	0.95	0.69, 1.31	289	233	1.33	0.98, 1.82	109	153	2.22	1.55, 3.17
AA	99	65	1.04	0.69, 1.56	118	93	1.32	0.91, 1.93	66	51	1.19	0.76, 1.87
p interaction	0.11											
LEP (rs2167270)												
GG	217	139	1.00		250	186	1.23	0.92, 1.64	111	113	1.59	1.13, 2.23
GA	243	159	1.07	0.79, 1.43	274	228	1.38	1.04, 1.83	144	144	2.07	1.48, 2.88
AA	73	29	0.63	0.39, 1.03	101	59	0.93	0.63, 1.37	40	49	1.87	1.16, 3.02
p interaction	0.08											

[1] Odds ratios and 95% confidence intervals adjusted for 5-year. age category, sex and race.

[2] Women recently exposed to estrogen are defined as those women who were premenopausal or who had taken HRT within the past 2 years.

TABLE III

Asssociation between Aspirin/NSAIDS use, Leptin (*LEP*) and Leptin Receptor (*LEPR*) and Colon Cancer Risk

Aspirin/NSAIDS	Aspirin/NSAID Use							
	Controls (*n*)	Cases (*n*)	OR[1] No current use	95% CI	Controls (*n*)	Cases(*n*)	OR Current use	95% CI
LEPR (rs6588147)								
AA	531	528	1.00		357	238	0.67	0.55, 0.83
AG	496	449	0.92	0.78, 1.10	354	206	0.60	0.48, 0.74
GG	129	109	0.88	0.78, 1.16	102	46	0.46	0.32, 0.67
p interaction	0.38							
LEP (rs7799039)								
GG	358	358	1.00		280	150	0.55	0.43, 0.70
GA	555	540	1.03	0.85, 1.25	391	249	0.67	0.54, 0.84
AA	250	191	0.82	0.64, 1.04	148	95	0.68	0.50, 0.91
p interaction	0.04							
LEP (rs2167270)								
GG	484	407	1.00		316	206	0.77	0.62, 0.96
GA	502	544	1.29	1.08, 1.55	367	225	0.74	0.59, 0.91
AA	171	135	0.93	0.71, 1.20	133	60	0.54	0.39, 0.75
p interaction	0.04							

[1] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, race and center.

TABLE IV

Associations between Insulin-Related Genes, Leptin (*LEP*), Leptin Receptor (*LEPR*) and Colon Cancer Risk

IGF1

Gene / Genotype	19/19				19/non 19				non 19/non 19			
	Controls (n)	Cases (n)	OR	95%CI	Controls (n)	Cases (n)	OR	95%CI	Controls (n)	Cases (n)	OR	95%CI
LEPR (rs6588147)												
AA	341	296	1.00		421	354	0.97	0.78, 1.20	114	109	1.00	0.73, 1.37
AG	352	264	0.87	0.70, 1.09	385	292	0.89	0.71, 1.10	102	89	1.02	0.73, 1.42
GG	101	62	0.72	0.50, 1.03	94	74	0.91	0.65, 1.29	28	18	0.78	0.42, 1.46
p interaction	0.55											
LEP (rs7799039)												
GG	232	192	1.00		294	229	0.93	0.71, 1.21	104	83	0.85	0.59, 1.23
GA	403	322	1.01	0.79, 1.29	436	359	1.03	0.81, 1.31	93	102	1.37	0.97, 1.93
AA	163	112	0.85	0.62, 1.16	176	134	0.97	0.72, 1.31	49	31	0.80	0.49, 1.31
p interaction	0.13											
LEP (rs2167270)												
GG	331	224	1.00		368	284	1.16	0.92, 1.46	87	91	1.48	1.04, 2.09
GA	358	321	1.37	1.09, 1.72	393	343	1.31	1.05, 1.64	103	101	1.43	1.03, 1.99
AA	108	76	1.02	0.72, 1.44	140	94	0.98	0.71, 1.34	53	24	0.63	0.38, 1.06
p interaction	<0.01											

IRS2

Gene / Genotype	GG				GD				GD/DD			
	Controls (n)	Cases (n)	OR	95%CI	Controls (n)	Cases (n)	OR	95%CI	Controls (n)	Cases (n)	OR	95%CI
LEPR (rs6588147)												
AA	380	357			495		1.00		406		0.90	0.74, 1.10
AG	343	287			499		0.92	0.74, 1.13	356		0.79	0.64, 0.96
GG	97	68			126		0.78	0.55, 1.10	85		0.75	0.55, 1.02
p interaction	0.88											
LEP (rs7799039)												
GG	274	226			357		1.00		273		0.99	0.78, 1.26
GA	384	363			550		1.24	0.98, 1.57	421		1.01	0.81, 1.27
AA	167	126			220		1.00	0.74, 1.34	155		0.93	0.70, 1.23
P interaction	0.44											
LEP (rs2167270)												
GG	358	282			427		1.00		323		0.98	0.79, 1.21
GA	334	356			523		1.36	1.10, 1.69	408		1.02	0.83, 1.25
AA	131	75			171		0.72	0.52, 0.99	116		0.88	0.66, 1.17
p interaction	0.04											

Odds rations (OR) and 95% confidence intervals (CI) adjusted for age category, sex and race.

TABLE V

Association between, *VDR*, Leptin (*LEP*) and Leptin Receptor (*LEPR*) and Colon Cancer Risk

VDR Fok1

	FF				Ff/ff			
	Controls (*n*)	Cases (*n*)	OR	95% CI	Controls (*n*)	Cases (*n*)	OR	95% CI
LEPR (rs6588147)								
AA	312	315	1.00		510	405	0.81	0.66, 0.99
AG	289	246	0.86	0.68, 1.08	515	375	0.74	0.60, 0.92
GG	84	56	0.68	0.46, 0.98	130	82	0.65	0.47, 0.90
p interaction	0.45							
LEP (rs7799039)								
GG	223	232	1.00		373	234	0.63	0.49, 0.81
GA	319	285	0.91	0.71, 1.16	568	457	0.82	0.66, 1.04
AA	145	103	0.73	0.53, 1.00	221	174	0.80	0.61, 1.06
p interaction	<0.01							
LEP (rs2167270)								
GG	292	228	1.00		444	353	1.04	0.83, 1.30
GA	297	293	1.27	1.00, 1.61	524	428	1.08	0.87, 1.34
AA	96	95	1.26	0.90, 1.76	189	83	0.57	0.42, 0.78
p interaction	<0.01							

VDR Bsml

	bb				bB/BB			
	Controls (*n*)	Cases (*n*)	OR[1]	95% CI	Controls (*n*)	Cases (*n*)	OR	95% CI
LEPR (rs6588147)								
AA	320	261	1.00		546	492	1.12	0.91, 1.38
AG	297	238	1.01	0.79, 1.28	539	400	0.93	0.76, 1.15
GG	68	63	1.17	0.80, 1.72	153	90	0.75	055, 1.02
p interaction	0.02							
LEP (rs7799039)								
GG	221	197	1.00		400	303	0.87	0.68, 1.11
GA	318	278	1.05	0.81, 1.35	610	497	0.97	0.77, 1.22
AA	152	89	0.70	0.50, 0.97	233	186	0.96	0.73, 1.26
p interaction	0.04							
LEP (rs2167270)								
GG	277	211	1.00		504	384	1.01	0.81, 1.27
GA	298	279	1.24	0.98, 1.59	551	478	1.16	0.94, 1.45
AA	113	70	0.82	0.58, 1.16	184	123	0.88	0.66, 1.18
p interaction	0.76							

[1] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, race and center.