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Interleukin-4 and interleukin-4 receptor polymorphisms and colorectal cancer risk

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ABSTRACT

Interleukin-4 (IL-4) and interleukin-4 receptor (IL-4R) modulate inflammation and are associated with the colorectal adenoma-carcinoma progression and the metastatic capacity. IL-4 also causes a dose-dependent reduction of proliferation in colorectal cancer cells. The aim of the study was to evaluate whether genetic variants within *IL4* and *IL4R* could affect the individual risk to develop colorectal cancer. We genotyped all the polymorphisms coding for an aminoacidic change in *IL4R* and we used a haplotype-tagging SNP approach for *IL4*. We carried out a case-control association study by genotyping, with the 5′ nuclease assay, two common SNPs within *IL4* (−588C > T, Ex1-168G > A) and five SNPs within *IL4R* (I75V, C431R, S436L, S503P, Q576R) in 377 cases of colorectal cancer and 326 controls from Spain. No statistically significant association between the SNPs investigated and colorectal cancer risk was found, as main effects. When the sub-analyses were carried out, the homozygotes for *IL4* −588C > T or for Ex1-168G > A showed an increased risk for colon cancer only, with the odds ratios of 4 (95% CI 0.97–16.6; *P*-interaction = 0.016 and 4.66 (95% CI 1.16–18.77; *P*-interaction = 0.023), respectively. Moreover, women showed a significant increased risk associated to the IL4 rare alleles and this was clearly greater than that in men (for Ex1-168G > A: OR = 1.96; 95% CI = 1.11–3.47; *P*-interaction = 0.006). However, when sub-groups are analysed, the findings should be taken with caution for the weakening of the statistical power.

1. Introduction

Interleukin-4 (IL-4) plays an important role in regulating the immune response of B cells, T cells, and macrophages against infections and malignant cells.[1–3] Both *IL4* and *IL4R* gene knockout mice show defective production of Th2 lymphocytes or reduced IgE levels.[4,5] Interestingly, in cancer cell lines, variations in the activity of IL-4 and its receptors were shown to modulate cell proliferation, and to affect signal transduction pathways.[6] In particular, in human colorectal cancer (CRC) cells, IL-4 and interleukin-4 receptor (IL-4R) are associated with the adenoma-carcinoma progression and

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with metastatic capacity.[7,8] IL-4 causes a dose-dependent reduction of proliferation[9] and triggers a decrease of matrix metalloproteinases (MMP-1, −2, and −9), a reduction of cell-matrix invasiveness, and the arrest of cell migration.[10,11] These findings also have important clinical implications, since IL-4 receptors are used as novel targets for cancer cytotoxin therapy, under testing in recent clinical trials.[12,13] Thus, in the present study, we evaluated whether single nucleotide polymorphisms (SNPs) within *IL4* and *IL4R* genes were associated with the risk to develop CRC and whether they could be associated with survival or response to therapy among cases. The possible interactions between polymorphisms and other relevant variables (including tumour location, dietary habits, use of non-steroidal anti-inflammatory drugs, NSAIDs) were also explored. In order to reach the goals, we performed a case–control study and genotyped two SNPs within *IL4* (−588C > T, dbSNP ID: rs2243250; Ex1-168G > A, rs2070874) and five SNPs within *IL4R* (I75V, rs1805010; C431R, rs1805012; S436L, rs1805013; S503P, rs1805015; Q576R, rs1801275) in 377 cases and 326 controls from Barcelona (Spain).

2. Materials and methods

2.1. Study design and case ascertainment

A hospital-based case–control study was conducted to assess gene–environment interactions in relation to CRC risk. Cases were patients with a new diagnosis of CRC attending a University Hospital in Barcelona, Spain, between January 1996 and December 1998. All cases had histological confirmation of their tumour diagnosis. During the study period, a total of 523 cases were diagnosed with sporadic CRC in the hospital. This study includes those 377 (72%) who could be interviewed and who provided biological samples of sufficient quality for genetic analysis. Refusals were 2%, while 14% could not be interviewed because they had either died, had mental or some other impairment, or were released without being approached and could not be traced. Finally, 12% were interviewed but did not provide biological samples. These lost cases were similar to those included with respect to age, sex, tumour location and extent.

Controls were randomly selected among patients admitted to the same hospital during the same period. To avoid selection bias, the criterion for inclusion of controls was that the reason for the current admittance to the hospital should be a new disease (not previously diagnosed) for that patient. This criterion was used to avoid inclusion of patients with chronic diseases, who might be repeatedly admitted to hospital and modify their habits because of their disease. This procedure paralleled the criterion for cases that were also newly diagnosed incident cases. Sex and broad age groups were used as stratifying criteria for frequency matching. Both cases and controls had to have good mental condition, and be able to see and hear and follow an interview. From the daily patient admission lists, candidate controls were approached and, if they met these criteria, they were invited to participate. Among 470 selected controls, a total of 326 (69.4%) were analysed in this study. Refusals were 7%, while 5% could not be interviewed because of mental or other impairment. Finally, 87 (18.6%) were interviewed but did not provide a blood sample. From a genetic point of view, we consider the hospitalised controls as being representative of the general Spanish population, as they came from very different hospital departments and included very different diseases. Moreover, it was recently reported in a study of 15,000 hospitalised controls that the frequencies of genetic polymorphisms did not differ from those of population controls.[14] No restriction criterion was imposed regarding the diagnosis of controls except those previously mentioned. The distribution of controls by diagnostic group was as follows: internal medicine 22%, acute surgery 19%, urology 17%, traumatology 15%, gastro-enterology 16%, circulatory or respiratory 11%. Sixty controls (18%) had a diagnosis of inflammatory conditions that might be related to the studied polymorphisms: inflammatory bowel disease ($N = 2$), peptic ulcer ($N = 2$), pancreatitis ($N = 24$), cholecystitis ($N = 16$), arthritis ($N = 12$) and diverticulitis ($N = 4$). We compared the distribution of genotypes in this group and found no difference from the rest of the controls. Also, an analysis of the data excluding these controls yielded essentially the same results. Thus, we decided not to exclude any control that had been selected.

All subjects were informed and gave written consent to participate in the study and to allow their biological samples to be genetically analysed, according to the Helsinki declaration. The Ethical Committee of the hospital cleared the study protocol.

2.2. Interviews and covariate assessment

Cases and controls were personally interviewed by trained personnel using a structured questionnaire to determine demographic characteristics and potential risk factors for CRC. For each subject, age and sex were recorded. A dietary history questionnaire, previously developed and validated in the framework of the EPIC study,[15] focused on average food consumption one year before the diagnosis of disease. The questionnaire recorded individual foods. For the analysis, post-hoc selected food groups (vegetables, fruits, etc.) were created by adding the consumption in grams of specific foods. Nutrient intakes were estimated with food composition tables developed *ad hoc* for the EPIC study.[16] To adjust for total energy intake, nutrient densities were calculated by dividing nutrient intake by total calories estimated from macronutrients plus alcohol. Supplemental vitamin intake is rare in this community and was not recorded. Self-reported weight and height were recorded for all individuals. Real weight and height were measured during the interview for 40% of the sample. Since the agreement between measured and reported values was very good (Pearson's $r = 0.97$), reported values were used for individuals if measurements were unavailable. Reported weight 10 years before interview was also recorded. Body mass index at that time was calculated assuming no change in height.

Lifelong long-term (at least six consecutive months) drug use was included in the questionnaire. An initial open question was followed by a list of 20 chronic diseases that are usually treated pharmacologically and their treatments were recorded. No drug list was used. For each exposure, the ages at initial use and cessation were recorded and cumulative

duration was computed. Drugs were grouped using the ATC (Anatomical Therapeutic Chemical) classification and the focus in this analysis was on NSAIDs. Other relevant risk factors explored were smoking, alcohol, and family history of cancer. For tobacco and alcohol, life-long history was recorded. We defined as an alcohol drinker a person consuming a minimum of one standard unit (equivalent to 10 g pure alcohol) per week. Average daily consumption and duration were calculated by summing the different exposure patterns during life.

2.3. Choice of polymorphisms under analysis

For *IL4R*, we firstly chose all the known variations coding for aminoacidic changes (the so-called 'cSNPs') (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=3566&chooseRs=coding). We assumed that an aminoacidic change could have a more relevant impact on the function of the receptor. Thus, we established a preliminary list of 12 polymorphisms, i.e.: rs1805010 (I75V), rs4787952 (G131V), rs6413500 (L387S), rs1805011 (A400E), rs1805012 (R431C), rs1805013 (L436S), rs1805015 (P503S), rs1801275 (R576Q), rs3024677 (I579V), rs3024678 (S675P), rs1805016 (A752S), and rs1805014 (P786S). Polymorphisms non validated or for which the reported minor allele frequency was below 10% (not allowing a sufficient statistical power) were discarded. The remaining seven SNPs (i.e.: I75V, A400E, R431C, L436S, P503S, R576Q, and A752S) were checked for their pair-wise linkage disequilibrium. The polymorphisms A400E and A752S resulted in complete linkage disequilibrium (according to www.hapmap.org, $r^2 = 1$) with R431C and L436S, respectively, and were thus eliminated because they provide redundant information. The final list of *IL4R* polymorphisms to be assayed was the following: I75V, C431R, S436L, S503P, and Q576R.

For *IL4* there are not any known cSNPs among Caucasians. In fact, according to SNP500Cancer Database, there is only one rare variant (C27R) present exclusively among Hispanics (http://snp500cancer.nci.nih.gov/snplist.cfm). Based on genotyping data from the SNP500Cancer Database, SNP rs2243290, as haplotype tagging SNP (ht-SNP), defines the two major haplotypes among Caucasians, having the frequencies of 83.2% and 10.3%. The analysis of this SNP alone misses only a third haplotype, having a frequency of 4.5%. For technical reasons (probe design), as htSNP, we preferred to genotype the polymorphism rs2070874 that is in complete linkage disequilibrium with rs2243290 (according to www.hapmap.org). Moreover, to enrich the analysis for *IL4*, we decided to genotype the most frequent polymorphism (according to SNP500Cancer Database) among the ones present in the promoter region, i.e. the rs2243250 (−588C > T).

2.4. SNP genotyping

Samples from cases and controls were randomised and mixed on PCR plates, so that an equal number of cases and controls could be analysed simultaneously. Genotyping was carried out with the 5′ nuclease assay by minor groove binder (MGB) probes fluorescently labelled with FAM or VIC and using the protocol recommended by the supplier (Applied Biosystems, Foster City, CA). Reactions were run in 96-well plates on a Tetrad DNA Engine PCR machine (MJ Research, Waltham, MA), and read in a TaqMan 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Probes and primers for the genotyping reaction are reported in Table 1.

2.5. Data analyses

Haplotypes were reconstructed with the software 'PHASE'.[17] Each polymorphism was tested in controls to ensure that it fits Hardy–Weinberg equilibrium. To test the hypothesis of association between genetic polymorphisms and CRC, multivariate methods based on logistic regression analyses were used. When cases were subdivided into groups (tumour site or stage), polytomous logistic regression was used, comparing each group of cases with the whole set of controls. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for each group compared to the class with the lowest level of exposure (set as having risk = 1). For polymorphisms, homozygosity for the more common allele among controls was set as the reference class. Tests for linear trend of ORs were calculated using the categorised variable as quantitative after assigning a linear score to each ordered category. Analyses were performed under both a co-dominant model (three genotypes separated) and an additive model (equivalent to a trend test on the number of minor alleles) to increase the statistical power. All analyses were adjusted for age and sex. The present study has a power of 80% to detect an OR of 2, when the prevalence of the minor genotype is 0.10 and 1.6 for a prevalence of 0.30. Analysis of the prognostic value of the polymorphisms was done using proportional hazards models adjusted for age, sex and tumour stage. The Kaplan–Meier method was used for the estimation of survival probabilities. More details concerning the statistical analyses were published previously.[18] To ensure quality control, DNA samples from cases and controls were randomly distributed on PCR plates, and all genotyping was conducted by personnel who were blinded to case–control status. Only genotype calls scored concordantly by two independent trained operators were retained. Finally, a random 8% of the samples were re-genotyped blindly.

3. Results

3.1. Main effects

The polymorphisms are in Hardy–Weinberg equilibrium in controls. The main effects related to each polymorphism by case/control status are shown in Table 2. The −588C > T and Ex1-168G > A polymorphisms of *IL4* and the I75V, C431R, S436L, S503P, Q576R polymorphisms of *IL4R* are not associated with the risk of colorectal cancer in this study. Haplotypes for *IL4* and *IL4R* did not associate with the risk of CRC as well (data not shown).

3.2. Interaction between polymorphisms and cancer site

Dividing cases for colon or rectal cancer sites, both *IL4* polymorphisms were associated with an increased risk that was statistically significant for the additive inheritance model. These results were driven by the associations of the homo-

Table 1 – PCR primer and TaqMan® probe sequences

Gene	Rs number	Trivial name	Primer forward	Primer reverse	VIC probe	6FAM probe
IL4	rs2243250	−588C > T	GATACGACCTGTCCTTCTCAAAACA	GCAGAATAACAGGCAGACTCTCCTA	AACATTGTCCCCCAGTG	CATTGTTCGCCCAGTGC
IL4	rs2070874	Ex1-168G > A	TGCATCGTTAGCTTCTCCTGATAA	ACCCATTAATAGGTGTCGATTTGC	TGTGAGGCAATTAG	CAATGTGAGACAATTA
IL4RA	rs1805010	I75V	CCTAACCCAGCCCCTGTGT	CCGCGCCTCCGTTGT	ACACGTGTATCCCTG	CGTGTGTCCCTGAGA
IL4RA	rs1805012	C431R	TGCCAGCAGGACATGGG	GGCATGTGAGCACTCGTACTTC	AGAAGGCATGACTCC	AAGGCGTGACTCC
IL4RA	rs1805013	S436L	AGCAGCAGGGATGACTTCCA	AGGTCCAGGAACAGGCTCTCT	TGCCCTCCCTTCC	AATGCCCGCCCTT
IL4RA	rs1805015	S503P	ACCCTGCTTACCGCAGCTT	GCTCTCTGGGACACGGTGACT	AGCAACTCCCTGAGC	CAGCAACCCCCTGAG
IL4RA	rs1801275	Q576R	CCTGCTCCACCGCATGTA	GAAATGTCCTCCAGCATGGG	TGGCTATCAGGAGTTT	TGGCTATCGGGAGTT

zygotes for the rare alleles. Homozygotes for −588C > T or for Ex1-168G > A showed an increased risk for colon cancer only, with the ORs of 4 (95% CI 0.97–16.6; *P*-interaction = 0.016) and of 4.66 (95% CI 1.16–18.77; *P*-interaction = 0.023), respectively. However, these associations were based on very few individuals, and they should be taken with caution. It should be noted that, for cases of colon cancer only, the haplotype that contained both rare alleles of *IL4* polymorphisms, was associated with a risk of 1.44, statistically not significant (95% confidence interval = 0.96–2.16; *P* = 0.082). Similar results were obtained when dividing the cases for tumour location in the left or right side of colon (data not shown).

3.3. *Interaction between polymorphisms and covariates*

The analyses for association based on interactions of *IL4* and *IL4R* polymorphisms with dietary variables (in particular: alcohol, tobacco, coffee, vegetables, meat and meat products, fats, fruits, BMI, calories) or consumption of aspirin or other NSAIDs did not elicit any statistical association (not shown). Interestingly, an interaction was observed between *IL4* polymorphisms and gender, as reported in Table 3. Women showed a significant increased risk associated to the *IL4* rare alleles and this was clearly greater than that in men (for Ex1-168G > A: OR = 1.96; 95% CI = 1.11–3.47; *P*–interaction = 0.006). This interaction remained significant when only colon cancer was analysed (data not shown). However, the numbers within each sub-group were too low and larger studies are warranted.

3.4. *Polymorphisms and prognosis*

Cases of CRC homozygous for rare alleles of *IL4* polymorphisms had a worse prognosis than patients with at least one common allele but association was not statistically significant (for −588C > T, HR = 2.1, 95%CI 0.7–6.9, *P* = 0.25 and for Ex1-168G > A, HR = 2.3, 95%CI 0.8–6.4, P = 0.15), considering that this group was very small (six cases for −588C > T and eight cases for Ex1-168G > A). This association was similar when disease free survival was analysed and no interaction was observed with tumour site, stage, or adjuvant chemotherapy. No relevant association was observed for any of the *IL4R* polymorphisms (data not shown).

4. Discussion

The functional meaning of the polymorphisms within *IL4R* is not completely understood. The genetic variations S503P and Q576R (falling in the intra-cytoplasmic domain of the receptor) were suggested to lead to loss and gain of function, respectively.[19] Previous association studies showed that I75V (also referred as I50V) is associated with rheumatoid arthritis,[20] susceptibility and severity of atopic asthma,[21] respiratory syncytial virus (RSV) bronchiolitis,[22] HIV infection and its progression to AIDS.[23] The variant S503P was associated with allergy and asthma,[24] atopy,[19] and affected the signal transduction pathway of STAT6, leading to a reduction of IgE concentrations.[25] Q576R have been associated with allergic asthma,[26,27] and mastocitosis.[28] According to these findings, it is conceivable that such genetic variations alter the properties of the receptor. Functional studies are warranted

Table 2 – Odds ratios and 95% confidence intervals for CRC by genotypes under investigation (main effects)

Gene and SNP		Controls	All cases		Rectal cancer		Colon cancer	
		N[a]	N[a]	OR (95%CI)[b]	N[a]	OR (95%CI)[b]	N[a]	OR (95%CI)[b]
IL4	−588C > T							
CC		209	214	1	83	1	131	1
CT		57	59	1.04 (0.68–1.58)	17	0.75 (0.41–1.37)	42	1.23 (0.77–1.95)
TT		3	7	2.63 (0.66–10.44)	1	0.84 (0.08–8.32)	6	4.00 (0.97–16.60)
Ptrend				0.39		0.38		0.08
IL4	Ex1-168G > A							
GG		209	221	1	88	1	133	1
GA		48	51	1.03 (0.66–1.61)	14	0.70 (0.37–1.35)	37	1.25 (0.76–2.05)
AA		3	9	3.35 (0.88–12.74)	2	1.67 (0.27–10.33)	7	4.66 (1.16–18.77)
Ptrend				0.24		0.55		0.047
IL4RA	I75V							
AA		81	83	1	29	1	54	1
AG		131	141	1.00 (0.67–1.49)	50	1.05 (0.61–1.80)	91	0.97 (0.62–1.52)
GG		51	55	0.98 (0.59–1.61)	24	1.26 (0.66–2.42)	31	0.83 (0.47–1.47)
Ptrend				0.93		0.50		0.55
IL4RA	C431R							
AA		208	235	1	85	1	150	1
AG		59	49	0.73 (0.48-1.12)	18	0.75 (0.42–1.35)	31	0.72 (0.44–1.18)
GG		2	2	0.68 (0.09-5.02)	2	2.15 (0.28–16.25)	0	0
Ptrend				0.14		0.60		0.099
IL4RA	S436L							
TT		202	221	1	81	1	140	1
TG		59	54	0.83 (0.55–1.27)	17	0.73 (0.40–1.32)	37	0.90 (0.56–1.44)
GG		5	5	0.80 (0.22–2.87)	3	1.38 (0.32–6.04)	2	0.48 (0.09–2.57)
Ptrend				0.38		0.56		0.42
IL4RA	S503P							
TT		181	201	1	74	1	127	1
TC		82	73	0.80 (0.55–1.17)	26	0.77 (0.46–1.30)	47	0.82 (0.54–1.26)
CC		3	7	1.80 (0.45–7.25)	4	3.02 (0.64–14.27)	3	1.16 (0.22–6.01)
Ptrend				0.54		0.89		0.45
IL4RA	Q576R							
AA		166	183	1	69	1	114	1
AG		90	87	0.87 (0.60–1.25)	31	0.83 (0.50–1.36)	56	0.90 (0.59–1.36)
GG		12	14	1.02 (0.46–2.30)	6	1.16 (0.42–3.24)	8	0.94 (0.37–2.39)
Ptrend				0.63		0.73		0.65

a For some polymorphisms, numbers do not sum up to the totals of controls (326) or cases (377) due to genotyping failure. All samples that did not give a reliable result in the first round of genotyping were resubmitted to additional genotyping. Data points that were still not filled after this procedure were left blank.

b OR = Odds ratio adjusted for age and sex; 95%CI = 95% confidence interval.

Table 3 – Odd ratios and 95% confidence intervals for the interaction between *IL4* polymorphisms and gender (dominant model)

Gene and SNP		Controls		Cases males		Cases females	
		N males	N females	N	OR (95% CI)	N	OR (95%CI)
IL4	−588C > T						
CC		105	104	141	1	80	1
CT + TT		26	25	26	0.76 (0.42–1.39)	34	1.78 (0.98–3.23)
Test for the interaction in the trend: $P = 0.0485$							
IL4	Ex1-168G > A						
GG		107	102	139	1	75	1
GA + AA		32	28	26	0.63 (0.36–1.13)	40	1.96 (1.11–3.47)
Test for the interaction in the trend: $P = 0.0060$							

to understand the functional impact of these polymorphisms on the biological activity of IL-4 receptor.

For *IL4*, previous association studies investigated the role of genetic variations not coding for aminoacidic changes. Recently, some of the haplotypes of *IL4* were associated with respiratory syncytial virus,[29] multiple sclerosis,[30] oral cancer,[31] and systemic lupus erythematosus,[32] indicating that there could be important polymorphisms affecting the regulation of this cytokine. However, a previous study failed to find any association between polymorphisms within *IL4* and colorectal adenomas, considered the pre-cancerous lesion for CRC.[33] Thus, the data presented in the present study, combined with the previous one, do not support a role of *IL4* and *IL4R* genes in the susceptibility to CRC. The heterozygous carriers of all the variants considered in this study are not at risk for CRC. According to Breslow and Day,[34] for a dominant model we had a 95% statistical power to detect a minimal OR of 2.2 using the rare polymorphism (the *IL4R*, C431R), and a minimal OR of 1.8 for the most frequent SNP (i.e. *IL4R*, I75V).

It is worth stressing that the rare homozygotes for both SNPs in *IL4* show a more than doubled risk of CRC but these ORs do not reach the statistical significance due to the small number of subjects. However, there might be an increased risk among women and especially for colon cancer, but not for rectal cancer. Both SNPs in *IL4* did not show a significant association with poor prognosis for individuals homozygous for the variants.

In summary, we could not detect an effect of the genetic variants within *IL4* and *IL4R* on colorectal cancer risk or overall survival in our population. We conclude that the genetic variants we have investigated are not relevant to sporadic colorectal cancer risk, or they have only an effect below the above mentioned statistical thresholds. More studies are warranted in the attempt to confirm the association between colon cancer and homozygosity of *IL4* polymorphisms among women.

Conflict of interest statement

None declared.

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REFERENCES

1. Tepper RI. The anti-tumour and proinflammatory actions of IL4. *Res Immunol* 1993;**144**(8):633–7.
2. Li-Weber M, Krammer PH. Regulation of IL4 gene expression by T cells and therapeutic perspectives. *Nat Rev Immunol* 2003;**3**(7):534–43.
3. Banchereau J. Converging and diverging properties of human interleukin-4 and interleukin-10. *Behring Inst Mitt* 1995:58–77.
4. Kopf M, Le Gros G, Bachmann M, et al. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 1993;**362**(6417):245–8.
5. Noben-Trauth N, Shultz LD, Brombacher F, et al. An interleukin 4 (IL-4)-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc Natl Acad Sci U S A* 1997;**94**(20):10838–43.
6. Beckmann MP, Cosman D, Fanslow W, et al. The interleukin-4 receptor: structure, function, and signal transduction. *Chem Immunol* 1992;**51**:107–34.
7. Kaklamanis L, Gatter KC, Mortensen N, Harris AL. Interleukin-4 receptor and epidermal growth factor receptor expression in colorectal cancer. *Br J Cancer* 1992;**66**(4):712–6.
8. Toi M, Bicknell R, Harris AL. Inhibition of colon and breast carcinoma cell growth by interleukin-4. *Cancer Res* 1992;**52**(2):275–9.
9. Lahm H, Schnyder B, Wyniger J, et al. Growth inhibition of human colorectal-carcinoma cells by interleukin-4 and expression of functional interleukin-4 receptors. *Int J Cancer* 1994;**59**(3):440–7.
10. Uchiyama A, Essner R, Doi F, et al. Interleukin 4 inhibits hepatocyte growth factor-induced invasion and migration of colon carcinomas. *J Cell Biochem* 1996;**62**(4):443–53.
11. Kanai T, Watanabe M, Hayashi A, et al. Regulatory effect of interleukin-4 and interleukin-13 on colon cancer cell adhesion. *Br J Cancer* 2000;**82**(10):1717–23.
12. Kawakami K, Kawakami M, Puri RK. Overexpressed cell surface interleukin-4 receptor molecules can be successfully targeted for antitumor cytotoxin therapy. *Crit Rev Immunol* 2001;**21**(1-3):299–310.
13. Eguchi J, Hiroishi K, Ishii S, et al. Interleukin-4 gene transduced tumor cells promote a potent tumor-specific Th1-type response in cooperation with interferon-alpha transduction. *Gene Ther* 2005;**12**(9):733–41.
14. Garte S, Gaspari L, Alexandrie AK, et al. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001;**10**(12):1239–48.
15. EPIC group of Spain. Relative validity and reproducibility of a diet history questionnaire in Spain. I. Foods, European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997;**26**(Suppl 1):S91.
16. EPIC group of Spain. Relative validity and reproducibility of a diet history questionnaire in Spain. II. Nutrients, European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997;**26**(Suppl 1):S100.
17. Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am J Hum Genet* 2005;**76**(3):449–62.
18. Landi S, Moreno V, Gioia-Patricola L, et al. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* 2003;**63**(13):3560–6.
19. Kruse S, Japha T, Tedner M, et al. The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. *Immunology* 1999;**96**(3):365–71.
20. Prots I, Skapenko A, Wendler J, et al. Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006;**54**(5):1491–500.
21. Hytonen AM, Lowhagen O, Arvidsson M, et al. Haplotypes of the interleukin-4 receptor alpha chain gene associate with

susceptibility to and severity of atopic asthma. *Clin Exp Allergy* 2004;**34**(10):1570–5.
22. Hoebee B, Rietveld E, Bont L, et al. Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor alpha polymorphisms. *J Infect Dis* 2003;**187**(1):2–11.
23. Soriano A, Lozano F, Oliva H, et al. Polymorphisms in the interleukin-4 receptor alpha chain gene influence susceptibility to HIV-1 infection and its progression to AIDS. *Immunogenetics* 2005;**57**(9):644–54.
24. Tam EK, Jourdan-LeSaux C, Stauder S, et al. Polymorphisms in the interleukin-4 receptor alpha chain: association with traits of allergy and asthma in an admixed population in Hawaii. *Cell Mol Biol (Noisy-le-grand)* 2003;**49**(8):1345–9.
25. Curiel RE, Lahesmaa R, Subleski J, et al. Identification of a Stat-6-responsive element in the promoter of the human interleukin-4 gene. *Eur J Immunol* 1997;**27**(8):1982–7.
26. Cui T, Wu J, Pan S, Xie J. Polymorphisms in the IL-4 and IL-4R [alpha] genes and allergic asthma. *Clin Chem Lab Med* 2003;**41**(7):888–92.
27. Risma KA, Wang N, Andrews RP, et al. V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function. *J Immunol* 2002;**169**(3):1604–10.
28. Daley T, Metcalfe DD, Akin C. Association of the Q576R polymorphism in the interleukin-4 receptor alpha chain with indolent mastocytosis limited to the skin. *Blood* 2001;**98**(3):880–2.
29. Puthothu B, Krueger M, Forster J, Heinzmann A. Association between severe respiratory syncytial virus infection and IL13/IL4 haplotypes. *J Infect Dis* 2006;**193**(3):438–41.
30. Suppiah V, Goris A, Alloza I, et al. Polymorphisms in the interleukin-4 and IL-4 receptor genes and multiple sclerosis: a study in Spanish-Basque, Northern Irish and Belgian populations. *Int J Immunogenet* 2005;**32**(6):383–8.
31. Tsai MH, Chen WC, Tsai CH, et al. Interleukin-4 gene, but not the interleukin-1 beta gene polymorphism, is associated with oral cancer. *J Clin Lab Anal* 2005;**19**(3):93–8.
32. Wu MC, Huang CM, Tsai JJ, et al. Polymorphisms of the interleukin-4 gene in chinese patients with systemic lupus erythematosus in Taiwan. *Lupus* 2003;**12**(1):21–5.
33. Gunter MJ, Canzian F, Landi S, et al. Inflammation-related gene polymorphisms and colorectal adenoma. *Cancer Epidemiol Biomarkers Prev* 2006;**15**(6):1126–31.
34. Breslow NE, Day NE. Statistical methods in cancer research, Volume II–The design and analysis of cohort studies. *IARC Sci Publ* 1987(82):1–406.