Pathol. Oncol. Res. (2010) 16:485–496
DOI 10.1007/s12253-010-9263-9

Estrogen Receptor Alpha Polymorphisms and the Risk of Malignancies

Andrei Anghel · Diana Narita · Edward Seclaman · Emilian Popovici · Mariana Anghel · Liviu Tamas

Received: 6 December 2009 / Accepted: 22 March 2010 / Published online: 11 April 2010

Abstract Estrogens represent risk factors for endocrine-related cancers and play also an important role in the development and progression of other malignancies. In order to analyze the associations between estrogen receptor gene alpha polymorphisms and cancers susceptibility, we genotyped six single nucleotide polymorphisms (SNPs) in 163 Caucasian cancer patients—103 breast cancers and 60 other malignancies (colorectal, bladder, hepatocellular carcinoma and acute myeloid leukemia)—and 114 healthy controls using hybridization probes. We performed Armitage`s association trend-test to evaluate the risk. Linkage disequilibrium (LD) was assessed for each pair of markers. The genotypes CC and CT of rs3798577 were significantly associated with the cancers risk (p-trend $_{breast}=4\times10^{-5}$; p-trend $_{cancers}=1\times10^{-5}$); in discrepancy with breast cancer where the C-allele represented the risk allele, for bladder, hepatocellular carcinomas and leukemia, the T allele seems to confer susceptibility. The minor G allele of rs1801132 was protective in our cases ($p=1\times10^{-4}$); for rs2228480, the heterozygous frequency was higher for cancer groups ($p=0.03$); the SNP pairs rs2228480&rs3798577 and rs2234693&rs9340799 were in low LD; the haplo types T-A of rs2234693&rs9340799 and G-C of rs2228480&rs3798577 showed a trend to be higher represented in breast cancers; T allele of rs2234693 was higher expressed in breast, colon cancers and leukemia; rs2077647 was associated with colon ($p=0.008$, C-risk allele) and bladder ($p=0.01$, T-risk allele) cancers. We concluded that ESR1 polymorphisms may have distinct impact in carcinogenesis and further genotyping will establish whether these findings remain significant in larger cohorts.

Keywords Acute myeloid leukemia · Bladder · Breast · Colorectal · Hepatocellular carcinomas · Susceptibility · Estrogen receptor alpha gene · Single nucleotide polymorphisms

Abbreviations

AML	acute myeloid leukemia
AR	androgen receptor
CRC	colorectal cancer
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
ESR1	estrogen receptor alpha gene
HCC	hepatocellular carcinoma
HRT	hormone replacement therapy
HWE	Hardy-Weinberg equilibrium
LD	linkage disequilibrium
MMR genes	mismatch repair genes
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism
UTR	untranslated region

A. Anghel · D. Narita (✉) · E. Seclaman · L. Tamas
Biochemistry Department,
University of Medicine and Pharmacy "Victor Babes" Timisoara,
Eftimie Murgu 2A,
Timisoara 300041, Romania
e-mail: diana_narita@yahoo.com
e-mail: biochim@umft.ro

E. Popovici · M. Anghel
Epidemiology Department,
University of Medicine and Pharmacy "Victor Babes" Timisoara,
Eftimie Murgu 2A,
Timisoara 300041, Romania

Introduction

Malignant transformation occurs through the accumulation of mutations in genes regulating cell division, apoptosis,

invasiveness or metastasis. To date, six genes were associated with high risk of breast cancer (BRCA1, BRCA2, TP53, PTEN, STK11 and CDH1) and four genes were associated with modest risk (PALB2, BRIP1, ATM and CHEK2). There are also some low penetrance genes which can be associated with an increased risk, such as: ESR1, CASP8, FGRF2, TOX3, MAP3K1, LSP1, 8q24, etc [1, 2]. For breast cancer, which is the most common malignancy in women in developed countries, BRCA1 and BRCA2 mutations account for 20–40% of breast cancer that clusters in families and less than 5% of breast cancer overall, and are associated with a risk of up to 60–85% for breast and ovarian cancer. In addition to this high risk in hereditary breast cancer, there are certain heritable syndromes associated with an increased breast cancer risk [3]. However, more than 50% of the genetic predisposition remains unexplained and more emphasis has been placed on single-nucleotide polymorphisms and especially on an empirical approach in which a minimal set of tagging SNPs that efficiently captures all the common genetic variation in a gene is assayed [4, 5]. At present, it is widely accepted that breast cancer is a complex disease determined by the combined effect of several or even many genetic variants [6]. Although the risk conferred by individual loci is rather small, some risk alleles seem to act multiplicatively and when associated with environmental factors, the risk becomes significant. Notably, none significant breast cancer susceptible SNPs was found in ATM, BRCA1, BRCA2, CHEK2, TP53, genes that are known to be associated with increased breast cancer risk [7]. There are strong evidences that the level of ESR1 transcription and interactions of ERα with cofactors influence the carcinogenesis and estrogens represent risk factors for endocrine-related cancers such as breast, ovarian and endometrium malignant tumors [8, 9].

On the other hand, ER-pathway seems to influence some other types of cancers, but as opposed to breast cancer, there are only few studies in this direction. For example, for the descending *colon and rectum*, a higher global risk is observed in men while women with multiple pregnancies exhibit a reduced risk; the risk of CRC is also reduced in potmenopausal women undergoing hormone replacement therapy (HRT). Estrogen deprivation was associated with an increased risk of microsatellite instability in the genes linked to DNA repair and administration of estrogens reduced the reoccurrence of malignant polyps in patients recovering after surgical removal of colon tumors [10, 11]. Progressive loss of ER expression has been observed in human colorectal tumors [12]. It was demonstrated that ligand-activated ERα recruits APC (Adenomatous polyposis coli, tumor suppressor gene) to the promoters in ER target genes and enhance the estrogen-dependent ERE transactivation [13]. In this way, polymorphisms or mutations in the receptor ligand binding domain (LBD) could abolish this interaction. Slattery et al. (2005) supposed that at least one of the major DNA *MMR* (mismatch repair) genes is estrogen-responsive and loss of estrogen results in loss in DNA *MMR* capacity [14].

ERs and Bladder Cancer Several lines of evidence have linked estrogen to bladder carcinogenesis. Although men are more likely to develop bladder cancer than women, when data are corrected for the influence of smoking, women appear to have an even greater risk than men. Moreover, women have a worse prognosis than men. It was suggested that increased expression of ERα may contribute to early induction of cyclin D1 and cyclin E during the cell cycle and result in dysregulated cell proliferation in bladder cancer cells [15, 16].

ERs and Hepatocellular Carcinoma Hepatitis B virus (HBV) genome possesses a glucocorticoid response element and the level of ERs in the cytosol of peripheral blood mononuclear cells was significantly lower in asymptomatic HBV carriers and patients with chronic hepatitis than in healthy controls [17]. Deng et al. (2004) demonstrated in Chinese population that subjects carrying ESR1 29 T/T (in exon 1 S10S) genotype had an increased susceptibility to persistent HBV infection compared to those bearing at least one 29C allele ($p<0.001$). Pro325 and Thr594 exert no influence [18]. Zhai Y et al. (2006) studied six ESR1 polymorphisms and found a statistically significant increased susceptibility to hepatocellular carcinoma associated with the homozygous alleles with a high number of TA repeats, T29C T/T genotype, and pvuII C/C genotype [19].

ER-alpha and Acute Myeloid Leukemia (AML) In 1982, Danel et al. demonstrated the stimulating effect of estrogens on the growth of the human leukemic cell line HL60 and also the inhibitory effect on the same cell line after the addition of tamoxifen [20]. By immunocytochemistry the presence of ERs was demonstrated in the peripheral and bone marrow blast cells of patients newly diagnosed with acute myeloid leukemia and especially in patients that demonstrated an unusual reciprocal translocation t(6;11)(q27;q23.3) involving the locus of the ESR1 gene on chromosome 6 (6q24-qter) [21]. ESR1 SNPs were linked to the lymphoma risk and oral contraceptive use and hormone replacement therapy were associated with lower lymphoma risk [22], but to our best knowledge the relationship between ERα SNPs and AML was not yet reported in the literature.

Some ESR1 SNPs were widely studied, especially in relationship with breast cancers, but despite of the large number of association studies, the results are not in concordance and the individual contribution of these polymorphisms to the pathogenesis of breast cancer is still

unclear. Furthermore, for other types of malignant tumors in which ER-signaling is implicated, the pathogenesis is even more unclear. Taken together, these facts actuate us to analyze the associations between ERα polymorphisms and the risk of malignancies. In this context, the aim of our study was to genotype six ESR1 gene polymorphisms in breast and other types of cancers (colorectal cancer, acute myeloid leukemia, hepatocellular carcinoma, bladder cancer), in order to search for associations between these polymorphisms and the risk of cancer. Taken in account that the pathogenesis could be different, we intended also to compare allele susceptibility in breast versus other types of cancers.

Patients and Methods

SNPs selection After searching public SNPs databases, such as dbSNP http://www.ncbi.nlm.gov/SNP/ and available literature, we choose six SNPs. The selection criteria were: 1) >10 % frequencies in the Caucasian population; 2) position in the gene or in the functional domains of the ERα protein and possible functional relevance; because the non-synonymous SNPs are rare and non-validated in the Caucasian population we selected silent SNPs located in coding or modulating regions of ESR1; 3) the results from the previous genetic studies. For SNPs prioritization, we accessed the SNPs function prediction FASTSNP [23] and PUPASUITE [24] websites. The positions of the selected SNPs are represented in Fig. 1.

Patients and healthy controls Hundred sixty three (163) unrelated Caucasian patients with cancers were included into the current study: 103 breast cancers, 12 hepatocellular carcinomas, 15 AML, 15 bladder cancers and 18 colon cancers. For data analysis, we constituted three groups of samples: sample 1 consisted of DNA samples from breast cancer patients (exclusively women), sample 2 included the DNA from the other types of cancers and sample 3 that included the total number of cancers (sample1+sample2). We genotyped also 114 healthy subjects without family history of any type of cancers (first-and second-degree relatives) and we constituted two control groups selected to match the patients groups by age and gender: one control group of 90 healthy women for comparison with sample 1 and the second control group of 90 women plus 24 men for comparison with the samples 2 and 3. Written informed consent for DNA analysis was obtained from all subjects and the Ethics Committee of our University approved the study.

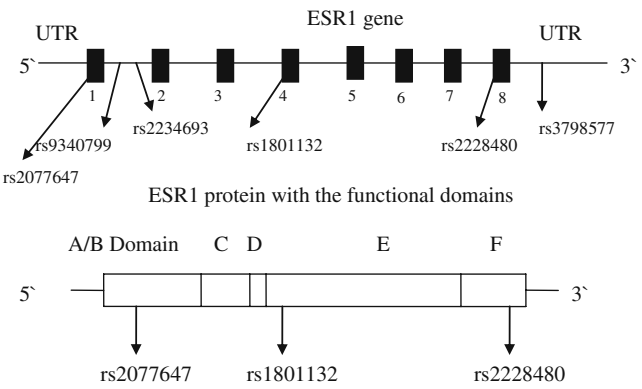

Fig. 1 The position of investigated SNPs on the gene, respectively on the ESR1 protein. The exons are represented with black boxes

SNPs genotyping Genomic DNA was isolated from 52 paraffin-embedded breast cancer tissue samples using RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Ambion—Applied Biosystems Foster City) and from 111 frozen cancer tissues using QiAmp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer protocol. In healthy subjects, the genomic DNA was extracted from whole blood collected on K_3EDTA using QiAmp DNA Blood kit (Qiagen, Hilden, Germany). For SNPs genotyping, we used hybridization probes. Two pairs of primers and probes were designed (TIB MOLBIOL GmbH, Berlin, Germany). The sequences of primers and probes that we used are shown in Table 1. All PCRs were performed in 0.2 ml thin-walled PCR tubes with 25 μl reactions mix on a Swift Maxi Instrument (Esco GB Ltd, Downton Wiltshire) using 30 ng of genomic DNA, with the forward and the reverse primers at 0.5 μM each and the probes at 0.3 μM each. PCR conditions were specific for each polymorphism and are resumed in Table 2. The amplification cycles were followed by melting cycles conducted in capillary tubes using LightCycler1.5 (Roche Applied Science, Roche Romania, Bucharest) in which DNA was denatured at 95°C for 30 s, cooled to 30°C using a rate of 1°C/s and held for 120 s. Temperature was then raised to 75°C with a transition rate of 0.1°C/s. Fluorescence was continuously monitored during the melt. Melting curves were converted into negative derivative curves of fluorescence with respect to temperature (dF/dT) by the LC (LightCycler) Data Analysis software (Roche Applied Science, Roche Romania, Bucharest). All analyses were performed with background correction and color compensation. As a negative control, the template DNA was replaced with PCR-grade water.

Data analysis Hardy-Weinberg equilibrium (HWE) was calculated for each SNP in each control group and samples separately, using Pearson's chi-square test. This step was performed also for patients, in order to determine whether there was any departure from HWE because such a finding can suggest that the marker is linked to a susceptible or protective allele. Chi-square test was also used to calculate the differences in the allele frequencies between cases and

Table 1 The sequences of primers and probes

Polymorphisms and target sequences	Sequences	Tm °C
rs2234693	Forward primer: TgCTCAgTCTCTACATgTTCCT	52,7
Target sequence: CAAATGTCCCAGC[C/T] GTTTTATGCTTTGTC	Reverse primer: TCCAgggTTATgTggCAAT	54,8
	Sensor: TgTCCCAgCCgTTTTATgC–FL	57,3
	Anchor:LC640-TTgTCTCTgTTTCCCAgAgACCCTgAg–PH	66,5
rs9340799	Forward primer: AgACTTAATgTTTTTgCAggAAT	53,1
Target sequence: TATTTTTCTTTCAC[A/G] TTTTCTGGTTTATTT	Reverse primer: CAAAATgAAATTAgCTggTTTCT	53,4
	Sensor: CAACTCCAgACCACACTCAgg-FL	57,3
	Anchor:LC640-TCTgggAAACAgAGACAAAgCATAAAACAgC–PH	66,5
rs2077647	Reverse primer: CAAAATgAAATTAgCTggTTTCT	53,4
Target sequence: CATCCCGGTAGGG[T/C] CTACGAAACCACACC	Sensor: CAACTCCAgACCACACTCAgg-FL	57,3
	Anchor:LC640-TCTgggAAACAgAGACAAAgCATAAAACAgC–PH	66,5
	Anchor:LC640-CCCTACTgCATCAgATCCAAgggAACg-PH	68,0
rs1801132	Forward primer:ACCTgTgTTTTCAgggATACgA	57,0
Target sequence: ATGCTGAGCCCCC[C/G] ATACTCTATTCCGA	Reverse primer:gCTgCgCTTCgCATTCTTAC	59,6
	Sensor:gCTgAgCCCCCCATACTCTA-FL	57,0
	Anchor:LC640-CCgAgTATgATCCTACCAgACCCTTCA-PH	63,4
rs2228480	Forward primer: CTgTgTCTTCCCACCTACAg	52,8
Target sequence: GTTTCCCTGCCAC[G/A] GTCTGAGAGCTCCCT	Reverse primer: gggTAAAATgCAgCAgggATT	58,8
	Sensor: TCCCTgCCACAgTCTgAgAgC–FL	61,5
	Anchor: LC640-CCCTggCTCCCACACggTTCAg–PH	69,3
rs3798577	Forward primer: CCTgAACTTgCAgTAAggTCA	54,7
Target sequence: GCTGAACAGTAC[T/ C] TGTGCAGGATTGTTG	Reverse primer: CCACCCTgAgCAAgTCT	51,9
	Sensor: gAACAgTACCTgTgCAggATT–FL	51,4
	Anchor: LC640-TTgTggCTACTAgAgAACAAgAgggAA-PH	61,1

[a] The minor allele is displayed second

controls and between the two patients group (breast cancer and the other types of cancers). For association analyses between single loci polymorphisms and cancers we performed logistic regression and Armitage`s trend-test using the HWE and association test calculator (http://ihg2.helmholtz-muenchen.de/cgi-bin). Considering the polymorphic alleles as "risk allele", we calculated odds ratios (OR) and 95% confidence intervals (CI). LD (linkage disequilibrium) between all possible pairs of loci, separately for controls and cases, was estimated with the help of two software: Genepop software package (version 1.2) (http://genepop.curtin.edu.au/) which use the Markov chain method to estimate the exact *p* value and Fisher exact test [25] and CubeX software (http://www.oege.org/software/cubex/) to calculate D` (standardized linkage disequilibrium coefficient) and r^2 (correlation coefficient) [26].

Table 2 PCR conditions for each polymorphism

SNP	Denaturation		Annealing		Extension	
	T°C	Time (s)	T°C	Time (s)	T°C	Time (s)
rs2234693	95	30	52	30	70	40
rs9340799	95	30	52	30	70	40
rs2077647	95	30	57	30	70	40
rs1801132	95	30	55	30	70	40
rs2228480	95	30	52	30	70	40
rs3798577	95	30	52	30	70	40

Results

ESR1 genotyping results and association tests The genotyping success rate for the six selected SNPs was between 93.33% and 99.03%. Among our controls, the allele and genotypes frequencies are presented in Tables 3, 4 and 5 and there are not significant differences from the previous findings in Caucasian population [27]. Within the two control groups we observed a significant difference for rs1801132 (p=0.003 for [1]↔[2]), allele 1(C) being more frequent (f_a1=0.87) in the group of 90 women comparative with the mixed group of 114 healthy subjects (f_a1= 0.75), suggesting that for this SNP, allele C could be more frequent in women than in men.

Table 3 Association between genotyped SNPs in ESR1 and breast cancer (breast cancer patients versus controls represented by healthy women)

SNP	Tests for deviation from Hardy-Weinberg equilibrium (HWE)		Tests for association (C.I.: 95% confidence interval)				
	Controls, n=90[a](genotypes frequencies and HWE for healthy women)	Sample 1, n=103[a](genotypes frequencies and HWE for breast cancer patients)	Allele freq. difference	Heterozygous	Homozygous	Recessive model	Armitage's trend test
rs3798577			Risk allele 2				
			[1]<->[2]	[11]<->[12]	[11+]<->[22]	[11]<->[12+22]	common odds ratio
	n11=30	n11=8	OR=2.29	OR=5.39	OR=7.50	OR=6.03	OR=2.62
	n12=41	n12=59	C.I=[1.52-3.45]	CI.=[2.24-12.95]	CI=[2.86-19.65]	CI=[2.59-14.05]	
	n22=18	n22=36	chi2=15.90	chi2=15.87	chi2=18.60	chi2=20.24	chi2=16.72
	p_{HWE}=0.56	p_{HWE}=0.016	$p=7\times10^{-5}$	$p=7\times10^{-5}$	p=2×10^{-5}	p=6.834e-06	$p=4\times10^{-5}$
rs2228480							
	n11=62	n11=61	OR=1.66	OR=1.98	OR=1.01	OR=1.94	OR=1.68
	n12=21	n12=41	C.I.=[0.95-2.89]	C.I.=[1.05-3.74]	C.I.=[0.06-16.61]	C.I.=[1.03-3.62]	
	n22=1	n22=1	chi2=3.29;	chi2=4.56;	chi2=0.0;	chi2=4.37	chi2=3.82
	p_{HWE} =0.59	p_{HWE} =0.03	p=0.06	p=0.03	p=0.99	p=0.03	p=0.050;
rs2077647							
	n11=34	n11=39	OR=1.08	OR=1.13	OR=1.16	OR=1.14	OR=1.08
	n12=40	n12=52	C.I.=[0.71-1.66]	C.I.=[0.61-2.10]	C.I.=[0.43-3.09]	C.I.=[0.63-2.05]	chi2=0.16;
	n22=9	n22=12	chi2=0.15	chi2=0.16	chi2=0.09	chi2=0.19	
	p_{HWE}=0.58	p_{HWE}=0.39	p=0.69	p=0.69	p=0.76	p=0.66	p=0.68
rs1801132							
	n11=65	n11=83	OR=0.75	OR=0.64	OR=2.35	OR=0.68	OR=0.87
	n12=23	n12=19	C.I.=[0.40-1.41]	C.I.=[0.32-1.28]	C.I.=[0.09-58.71]	C.I.=[0.34-1.34]	chi2=0.84;
	n22=0	n22=1	chi2=0.77;	chi2=1.55;	chi2=0.78;	chi2=1.23;	
	p_{HWE}=0.16	p_{HWE}=0.9	p=0.38	p=0.21	p=0.37	p=0.26	p=0.36;
rs9340799	n11=38	n11=44	OR=0.89	OR=1.03	OR=0.49	OR=0.96	OR=0.82
	n12=45	n12=54	C.I.=[0.58-1.37]	C.I.=[0.57-1.86]	C.I.=[0.13-1.81]	C.I.=[0.54-1.71]	chi2=0.31;
	n22=7	n22=4	chi2=0.25;	chi2=0.01;	chi2=1.16;	chi2=0.02;	
	p_{HWE}=0.2	p_{HWE}=0.01	p=0.61	p=0.90	p=0.28	p=0.89	p=0.57
rs2234693	n11=37	n11=32	OR=0.93	OR=1.98	OR=0.31	OR=1.5	OR=0.8
	n12=38	n12=65	C.I.=[0.61-1.41]	C.I.=[1.06-3.67]	C.I.=[0.09-1.02]	C.I.=[0.83-2.72]	chi2=0.13;
	n22=15	n22=4	chi2=0.11;	chi2=4.70;	chi2=3.95;	chi2=1.83;	
	p_{HWE}=0.33	p_{HWE}=7×10^{-5}	p=0.74	p=0.03	p=0.04	p=0.17	p=0.72

Legend: rs3798577: T=allele 1; rs2228480: G=allele 1; rs2077647: T=allele 1; rs1801132: C=allele 1; rs2234693: C=allele 1

The following equations correspond to risk allele 2: Odds ratio (allele freq. difference) : (Case_a2 × Control_a1) / (Case_a1 × Control_a2); Odds ratio (heterozygous): (Case_12 × Control_11) / (Case_11 × Control_12);Odds ratio (homozygous) : (Case_22 × Control_11) / (Case_11 × Control_22); Odds ratio (allele positivity): ((Case_12+Case_22) x Control_11) / (Case_11 × (Control_12 + Control_22)) ; Common odds ratio: (Case_12 × Control_11/N01 + Case_22 × Control_12/N12 + 4 × (Case_22 × Control_11/N02)) / (Case_11 × Control_12 / N01 + Case_12 × Control_22/N12 + 4 × (Case_22 × Control_11× Case_11× Control_22) x 0.5/N02)

p_{HWE}=p value for HWE (HWE was calculated for each SNP in each control group and samples separately, using Pearson's chi-square test; HWE was not respected when $p_{HWE}<0.05$); departures from HWE can suggest a susceptible or protective allele

n11=number of homozygotes for the allele 1; n12=number of heterozygotes; n22=number of homozygotes for the risk allele 2

[a] The genotyping success rate for the six selected SNPs was between 93.33% and 99.03%

The observed genotype frequencies showed that all six genotyped SNPs were in HWE proportions in the control groups. The p values are presented in Tables 3 and 5. In the cases groups, SNP rs2228480 (p=0.03), rs3798577 (p=0.01), rs2234693 (p=0.00007) and rs9340799 (p=0.01) did not respect Hardy-Weinberg proportions for the breast cancer cases; for sample 2 (other cancers, without breast cancer), none SNP deviated from Hardy-Weinberg proportions; for the sample 3 (total number of cancers), rs2234693 (p=0.0001) and rs9340799 (p=0.01) did not respect HWE.

Associations with Breast Cancer (Sample 1 Versus Controls) Table 3 presents the genotypes and allele frequencies within the controls and breast cancer patients group, indicating that: in breast cancer patients, for rs3798577 the frequency of the common allele 1 (T) is significantly lower in patients ($p=7\times10^{-5}$), CT ($p=7\times10^{-5}$) and CC($p=2\times10^{-5}$) representing the susceptible genotypes (p-trend=4×10^{-5}). For rs2228480, it was a trend for allele 1 (G) to be less represented in patients (p=0.06); the heterozygous (GA) were significantly more represented in cases (p=0.03). For rs2234693, the majority of our breast cancer cases were heterozygous (p=0.03).

Associations with the Other Types of Cancers (Sample 2 Versus Controls) Table 4 shows the genotypes frequencies for every type of malignant tumor. Comparing these tumor cases with healthy controls, the results showed that the frequency for the allele 1 was significantly lower for rs3798577 (p=0.02) (the polymorphic allele C confers susceptibility), respectively significantly higher for rs1801132 (the polymorphic allele G is protective). For the other three loci, the frequency of genotypes for samples 2 and 3 was not significantly different in comparison with the controls. In colon cancers, allele 1 in rs2077647 was significantly less frequent (p=0.008) and it was a trend for allele 1 of rs1801132 to be more represented in cases (p=0.07) (allele G of rs1801132 seems to be protective). In bladder cancers, allele 1 of rs3798577 (p=0.009) respectively of rs2228480 (p=0.05 for allele 1 versus allele2 and p=0.004 for heterozygous) were the risk alleles, while allele 1(C) in rs2077647 was protective (p=0.01), contrarily with the colon cancers, where C allele was the risk allele. For AML, allele 1 (T) of rs2234693 was higher represented (p=0.02). For hepatocellular carcinomas we did not observe significant differences over healthy controls. For the sample 3 (total number of cancer cases) we observed significant differences for SNPs rs3798577 and rs1801132, the risk allele being the polymorphic allele C ($p=2\times10^{-5}$), respectively the common allele C ($p=1\times10^{-4}$) (Table 6).

Association Between Breast Cancer and Other Cancers (Sample 1 Versus Sample 2) For rs3798577, the genotype 22 (CC) was less frequent in the other malignant tumors

Table 4 Genotypes frequencies in the other types of cancer patients and controls

SNP	Genotype	Patients					Controls
		Bladder Cancer n=15(%)	Colon Cancer n=18(%)	AML n=15 (%)	Hepato-Carcinoma n=12 (%)	Total n=60 (%)	n=114 (%)
rs9340799 (A/G)	AA	9(60)	5(27.7)	10(66.7)	4(33.3)	28(46.7)	48(42.1)
	AG	6(30)	11(61.2)	5(33.3)	6(50)	28(46.7)	57(50)
	GG	0	2(11.1)	0	2(16.7)	4(6.7)	9(7.9)
	CC	9(60)	3(16.7)	10(66.7)	4(33.3)	26(43.3)	48(42.1)
rs2234693 (C/T)	CT	6(30)	13(72.2)	5(33.3)	6(50)	30(50)	48(42.1)
	TT	0	2(11.1)	0	2(16.7)	4(6.7)	18(15.8)
	TT	13(86.6)	5(27.8)	7(46.6)	4(33.34)	29(48.33)	48(42.1)
rs2077647 (T/C)	CT	1(6.6)	6(33.3)	8(53.4)	7(58.33)	22(36.67)	57(50)
	CC	1(6.6)	7(39)	0	1(8.33)	9(15)	9(7.9)
	CC	10(67)	14(77.9)	11(73.3)	7(58.33)	42(70)	63(55.3)
rs1801132 (C/G)	GC	5(33)	2(11.1)	4(26.67)	5(41.67)	18(30)	46(40.3)
	GG	0	0	0	0	0	5(4.4)
	GG	5(33.34)	15(83.4)	10(67)	10(83.33)	40(66.67)	77(67.6)
rs2228480 (G/A)	GA	10(67)	3(16.6)	5(33.3)	2(16.67)	20(33.34)	32(28)
	AA	0	0	0	0	0	5(4.4)
	TT	0	4(22.2)	5(33.3)	4(33.34)	13(21.66)	38(33.3)
rs3798577 (T/C)	TC	10(67)	9(50)	5(33.3)	5(41.66)	29(48.33)	57(50)
	CC	5(33)	5(27.8)	5(33.3)	3(0.25)	18(30)	19(16.7)

Table 5 Association between genotyped SNPs for other cancers (without breast cancers) (other cancers versus healthy controls)

SNP	Tests for deviation from HWE		Tests for association (C.I.: 95% confidence interval)				
	Controls, n=114[a](genotypes frequencies and HWE for healthy subjects)	Sample 2, n=60[a](genotypes frequencies and HWE for other cancers)	Allele freq. difference	Heterozygous	Homozygous	Allele positivity	Armitage's trend test
rs3798577			Risk allele 2				
			[1]<->[2]	[11]<->[12]	[11+]<->[22]	[11]<->[12+22]	common odds ratio
	n11=38	n11=13	OR=1.65	OR=1.48	OR=2.77	OR=1.80	OR=1.66
	n12=57	n12=29	C.I.=[1.06 -2.58]	C.I.=[0.68-3.22]	C.I.=[1.12-6.81]	C.I.=[0.87-3.74]	chi2=4.92;
	n22=19	n22=18	chi2=4.95;	chi2=1.02;	chi2=5.04;	chi2=2.58;	
	p_{HWE}=0.76	p_{HWE}=0.83	p=0.02	p=0.31	p=0.02	p=0.11	p=0.02
rs2228480	n11=77	n11=40	OR=0.88	OR=1.20	OR=0.17	OR=1.04	OR=0.97
	n12=32	n12=20	C.I.=[0.49-1.59]	C.I.=[0.61-2.36]	C.I.=[0.01-3.22]	C.I.=[0.53-2.02]	chi2=0.17;
	n22=5	n22=0	chi2=0.17;	chi2=0.29;	chi2=2.54;	chi2=0.01;	
	p_{HWE}=0.48	p_{HWE}=0.12	p=0.68	p=0.59	p=0.11	p=0.90	p=0.68
rs2077647	n11=48	n11=29	OR=1.02	OR=0.64	OR=1.65	OR=0.77	OR=1.13
	n12=57	n12=22	C.I.=[0.63-1.63]	C.I.=[0.32-1.25]	C.I.=[0.58-4.64]	C.I.=[0.41-1.45]	chi2=0.01;
	n22=9	n22=9	chi2=0.01;	chi2=1.71;	chi2=0.93;	chi2=0.62;	
	p_{HWE}=0.15	p_{HWE}=0.17	p=0.93	p=0.19	p=0.33	p=0.43	p=0.93
rs1801132	n11=63	n11=42	OR=0.54	OR=0.58	OR=0.13	OR=0.53	OR=0.52
	n12=46	n12=18	C.I.=[0.30-0.97]	C.I.=[0.30-1.14]	C.I.=[0.01-2.52]	C.I.=[0.27-1.02]	chi2=4.76;
	n22=5	n22=0	chi2=4.29;	chi2=2.45;	chi2=3.24;	chi2=3.57;	
	p_{HWE}=0.34	p_{HWE}=0.17	p=0.03	p=0.11	p=0.07	*p=0.058*	p=0.03
rs9340799	n11=48	n11=28	OR=0.87	OR=0.84	OR=0.76	OR=0.83	OR=0.86
	n12=57	n12=28	C.I.=[0.542-1.41]	C.I.=[0.44-1.61]	C.I.=[0.21-2.70]	C.I.=[0.44-1.55]	chi2=0.35
	n22=9	n22=4	chi2=0.30	chi2=0.27	chi2=0.18	chi2=0.33	
	p_{HWE}=0.15	p_{HWE}=0.38	p=0.58	p=0.60	p=0.67328	p=0.56	p=0.55
rs2234693	n11=48	n11=26	OR =0.79	OR=1.15	OR=0.41	OR=0.95	OR=0.74
	n12=48	n12=30	C.I.=[0.49-1.27]	C.I.=[0.59-2.23]	C.I.=[0.12-1.34]	C.I.=[0.50-1.78]	chi2=0.91
	n22=18	n22=4	chi2=0.92	chi2=0.18	chi2=2.27	chi2=0.02	
	p_{HWE}=0.30	p_{HWE}=0.22	p=0.33	p=0.67	p=0.13	p=0.87	p=0.34

Legend: rs3798577: T=allele 1; rs2228480: G=allele 1; rs2077647: T=allele 1; rs1801132: C=allele 1; rs2234693: C=allele 1

The following equations correspond to risk allele 2: Odds ratio (allele freq. difference) : (Case_a2 × Control_a1) / (Case_a1 × Control_a2); Odds ratio (heterozygous): (Case_12 × Control_11) / (Case_11 × Control_12);Odds ratio (homozygous) : (Case_22 × Control_11) / (Case_11 × Control_22); Odds ratio (allele positivity): ((Case_12+Case_22)×Control_11) / (Case_11 × (Control_12 + Control_22)) ; Common odds ratio: (Case_12 × Control_11/N01 + Case_22 × Control_12/N12 + 4×(Case_22 × Control_11/N02)) / (Case_11 × Control_12 / N01 + Case_12 × Control_22/N12 + 4×(Case_22 × Control_11× Case_11× Control_22)×0.5/N02)

p_{HWE}=p value for HWE (HWE was calculated for each SNP in each control group and samples separately, using Pearson's chi-square test; HWE was not respected when p_{HWE}<0.05); departures from HWE can suggest a susceptible or protective allele

n11=number of homozygotes for the allele 1; n12=number of heterozygotes; n22=number of homozygotes for the risk allele 2

[a] The genotyping success rate for the six selected SNPs was between 93.33% and 99.03%.

than in breast cancers (p=0.02) and as opposite with breast cancer, where allele 2 (C) confers susceptibility, for hepatocellular carcinomas (p=0.02) and AML (p=0.03), the common allele 1 (T) was the risk allele. Comparing breast cancers with each other type of malignancy separately, we observed that in colon cancers, allele 1 of rs2077647 was less represented (p=0.03); for rs2228480, allele 1 shows a trend to be higher represented (p=0.07) than in breast cancer cases; in bladder cancers, the common allele T for rs2077647 was higher represented (p=0.003), suggesting that, in bladder cancers allele T represents the risk allele.

Linkage disequilibrium analysis. Across all populations, we estimated LD for each pair of loci using two different softwares, CubeX software for pair-wise values D` and r^2 and Genepop software to estimate the p value and Fisher exact test. Within controls, using Genepop software, all possible pairs between the six genotyped SNPs were in linkage disequilibrium (Fisher's exact tests $p<0.0001$). Using CubeX, only the pairs between SNPs 1,2,3 and 4 were in low LD (D'=1.0 and r^2 range between 0.68 and 0.307); rs2234693 and rs9340799 were in LD each other but not with the other genotyped SNPs. For the total number of cases, in low LD were the pairs: rs2228480&rs3798577 (p=0.03), rs2228480 & rs9340799 (p=0.04), rs2077647 & rs9340799 ($p=2\times10^{-5}$), rs2077647 & rs2234693 ($p=2\times10^{-5}$) and rs2234693 & rs9340799 ($p=2\times10^{-5}$). Checking the pair-wise LD parameters, only the last pair of loci (rs2234693 & rs9340799) remains in LD (D`=1; $r^2$=0.8). For the breast cancer cases, only rs2234693&rs9340799 pair was in medium LD with both softwares (D`=1, r^2=0.76). Exploring the haplotype frequencies for the pairs which were in LD, we did not find statistically evident susceptible haplotypes although, the haplotype T-A of rs2234693&rs9340799 (f_{11}=0.63) and G-C of rs2228480&rs3798577 (f_{21}=0.448) showed a trend to be higher represented (p=0.09 respectively p=0.06) in breast cancer patients.

Discussions

The most widely studied polymorphisms of ESR1are the *pvu*II (T397C) (rs2234693) and *xba*I (A351G) (rs9340799) located in intron 1, separated by 50 base pairs. *Xba*I and *pvu*II polymorphisms were previously associated with breast and prostate cancer and also with bone mineral density, age at menopause, spontaneous abortions, HRT, colon and urotelial cancers, cardiovascular and Alzheimer's diseases, hepatitis B and the risk for hepatocellular carcinoma [1, 18, 28]. It was suggested that the polymorphic allele T was associated with increased levels of androstendione [29]. Possible functional mechanisms attributed to these polymorphisms include a change of ERα gene expression by altering the binding of transcriptional factors and influence on alternative splicing of ERα gene. The first intron in a gene, like the promoter, usually contains a larger number of regulatory sequences than other introns. However, the results are still conflicting and the molecular mechanism by which these polymorphisms influence receptor activity are yet unclear. It was noted that the T→C transition is associated with the loss of the *pvu*II restriction site results in a potential binding site for *myb* transcription factors that, in the presence of *b-myb*, is capable of augmenting in vitro the transcription of a downstream reporter construct 10-fold [30]. Thus, the presence of polymorphic allele might amplify ERα transcription. An alternative explanation is that the two polymorphisms in intron 1 may be in linkage disequilibrium with causal synonymous polymorphisms elsewhere in the ERα or another gene. In this regard, it has been established that intron 1 polymorphisms are in linkage disequilibrium with the upstream TA and GT repeats polymorphism in the promoter of ESR1, which were associated with microsatellite instability [31]. Searching in FASTSNP SNPs function prediction web, we found that rs9340799 (*xba*I) is an intronic enhancer, representing a binding site for the helix-loop-helix transcription factor Th1/E47 (G allele) and rs2234693 (*pvu*II) serves as transcription binding sites for v-myb (C allele) and SRY (T allele).

Several studies investigated the *pvu*II and *xba*I polymorphisms for possible association with CRC. For example, in a case-control study, Speer et al. (2001) found an association between the *Pvu*II polymorphisms and rectal cancer but not with *Xba*I. The P allele was found in 70% of the patients whereas in the controls the frequency was 50% (p=0.0015) but the difference was not directly associated with the expression of *erbB-2* and *EGFR* oncogenes [32]. It was also suggested that the presence of *Pvu*II p allele and *Xba*I x allele increase the risk of developing microsatellites instability in CRC patients [33, 34]. Slattery et al. (2005) evaluated genetic variants of the ERα (xbaI), ERβ (CA repeat) and AR (CAG repeat) genes and associated increasing number of CA repeats and CAG repeats with the risk of colon cancer among women and respectively, among men; *Xba*I was not significantly associated [14]. In our cases, we did not observe differences for *Xba*I, but for *Pvu*II, the heterozygous were higher represented for CRC (p=0.02), breast cancers (p=0.03) and for AML, the T allele was significantly higher expressed (p=0.02). In accordance with the foregoing publications [28, 35–37], in our samples the two SNPs were in LD. The haplotype T-A (11) was more frequent in cancers cases, but the difference was not statistically significant (p=0.06). For breast cancers, regarding these markers, the results from literature are divergent,

Table 6 Association for the total number of investigated cancers: cancers (n=163) versus healthy controls (n=114, healthy subjects)

SNP	Tests for deviation from HWE		Tests for association (C.I.: 95% confidence interval)				
	Controls, n=114[a] (genotypes frequencies and HWE for healthy subjects)	Sample 3, n=163[a] (genotypes frequencies and HWE for cancers)	Allele freq. difference	Heterozygous	Homozygous	Allele positivity	Armitage trend test
rs3798577			Risk allele 2				
			[1]<->[2]	[11]<->[12]	[11+]<->[22]	[11]<->[12+22]	Common odds ratio
	n11=38	n11=21	OR=2.11	OR=2.79	OR=5.14	OR=3.38	OR=2.26
	n12=57	n12=88	C.I.=[1.49-2.97]	C.I.=[1.49-5.23]	C.I.=[2.43-10.85]	C.I.=[1.85-6.17]	chi2=19.28
	n22=19	n22=54	chi2=18.33	chi2=10.62	chi2=19.59	chi2=16.74	
	p_{HWE}=0.76	p_{HWE}=0.10	$p=2\times10^{-5}$	$p=1.12\times10^{-3}$	p=9.603e-06	$p=4\times10^{-5}$	$p=1\times10^{-5}$
rs2228480	n11=77	n11=101	OR=1.06	OR=1.45	OR=0.15	OR=1.27	OR=1.002
	n12=32	n12=61	C.I.=[0.68-1.63]	C.I.=[0.86-2.44]	C.I.=[0.01-1.33]	C.I.=[0.77-2.11]	chi2=0.08
	n22=5	n22=1	chi2=0.07	chi2=1.99	chi2=3.77	chi2=0.91	
	p_{HWE}=0.48	p_{HWE}=0.01	p=0.78	p=0.15	p=0.052	p=0.34	p=0.78
rs2077647	n11=48	n11=46	OR=1.17	OR=0.89	OR=1.85	OR=1.02	OR=1.25
	n12=57	n12=49	C.I.=[0.79-1.72]	C.I.=[0.51-1.56]	C.I.=[0.74-4.61]	C.I.=[0.60-1.74]	chi2=0.67
	n22=9	n22=16	chi2=0.64	chi2=0.15	chi2=1.80	chi2=0.01	
	p_{HWE}=0.15	p_{HWE}=0.61	p=0.42	p=0.70	p=0.18	p=0.91	p=0.41
rs1801132	n11=63	n11=125	OR=0.41	OR=0.40	OR=0.10	OR=0.37	OR=0.38
	n12=46	n12=37	C.I.=[0.26-0.65]	C.I.=[0.23-0.68]	C.I.=[0.01-0.88]	C.I.=[0.22-0.63]	chi2=15.85
	n22=5	n22=1	chi2=14.99	chi2=11.50	chi2=6.34	chi2=14.12	
	p_{HWE}=0.34	p_{HWE}=0.32	p=0.0001	p=0.0007	p=0.01	p=0.0001	$p=7\times10^{-5}$
rs9340799	n11=48	n11=72	OR=0.88	OR=0.9	OR=0.593	OR=0.9	OR=0.83
	n12=57	n12=82	C.I.=[0.61-1.27]	C.I.=[0.58-1.57]	C.I.=[0.21-1.64]	C.I.=[0.56-1.47]	chi2=0.53
	n22=9	n22=8	chi2=0.44	chi2=0.03	chi2=1.03	chi2=0.15	
	p_{HWE}=0.15	p_{HWE}=0.01	p=0.50	p=0.86	p=0.31	p=0.69	p=0.46
rs2234693	n11=48	n11=58	OR=0.9	OR=1.63	OR=0.36	OR=1.292	OR=0.79
	n12=48	n12=95	C.I.=[0.63-1.28]	C.I.=[0.977-2.745]	C.I.=[0.14-0.92]	C.I.=[0.79-2.11]	chi2=0.38
	n22=18	n22=8	chi2=0.33	chi2=3.53	chi2=4.79	chi2=1.04	
	p_{HWE}=0.30	p_{HWE}=0.0001	p=0.56	p=0.060	p=0.02	p=0.30	p=0.54

Legend: rs3798577: T=allele 1; rs2228480: G=allele 1; rs2077647: T=allele 1; rs1801132: C=allele 1; rs2234693: C=allele 1; The following equations correspond to risk allele 2: Odds ratio (allele freq. difference) : (Case_a2 × Control_a1) / (Case_a1 × Control_a2); Odds ratio (heterozygous): (Case_12 × Control_11) / (Case_11 × Control_12);Odds ratio (homozygous) : (Case_22 × Control_11) / (Case_11 × Control_22); Odds ratio (allele positivity): ((Case_12+Case_22)×Control_11) / (Case_11 × (Control_12 + Control_22)) ; Common odds ratio: (Case_12 × Control_11/N01 + Case_22 × Control_12/N12 + 4 × (Case_22 × Control_11/N02)) / (Case_11 × Control_12 / N01 + Case_12 × Control_22/N12 + 4×(Case_22 × Control_11× Case_11× Control_22)×0.5/N02)

p_{HWE}=p value for HWE (HWE was calculated for each SNP in each control group and samples separately, using Pearson's chi-square test; HWE was not respected when $p_{HWE}<0.05$); departures from HWE can suggest a susceptible or protective allele; n11=number of homozygotes for the allele 1; n12=number of heterozygotes; n22=number of homozygotes for the risk allele 2

[a] The genotyping success rate for the six selected SNPs was between 93.33% and 99.03%

some publications found no significant risk [36, 38–40], other found risk only for haplotypes [41] and yet other publications found an increased risk for the polymorphic alleles [42, 43] or in contrary for the wild type [44].

rs2077647 (C/T) is a silent polymorphism located in exon 1 (S10S). This location corresponds to the A/B structural domain, respectively TAF1 functional domain (ligand independent transactivation domain) and can be activated via the non-genomic pathway through compounds like AMPc, dopamine, growth factors like IGF and EGF, resulting in activation of the kinases pathways (MAPK/p38, PLC/PKC, JAK/STAT). The effects that are mediated by this mechanism are induced quickly (seconds or minutes) and regulate numerous cellular processes from proliferation and apoptosis to differentiation function of target cells. The A/B domain contains a co-regulator domain that binds co-activators or co-repressors to the ER, with an important role for the modulation of ER transcription.

We associated the T allele of this SNP with an increased risk of bladder cancer (p=0.01); in colon cancer, as opposed to bladder cancer, the C allele seems to be the risk allele (p=0.008). The ERs polymorphisms have not been studied yet in bladder cancers but, in renal cell carcinoma, Tanaka et al. 2003 studied six polymorphic loci on ERalpha gene (codon 10 T/C, codon 87 G/C, codon 243 C/T, codon 325 C/G, codon 594 G/A, pvuII, xbaI) for 113 patients and demonstrated that S10S polymorphism may be involved in renal cancer risk [45]. For breast cancer, in accordance with other studies on Caucasian population [46, 47] our results did not show significant differences over the control group for this marker. In Taiwanese population it was found an increased risk for breast cancer in the presence of allele T [48].

rs1801132 is a coding-synonymous, CG SNP in codon 325 (325Pro) of exon 4 of ESR1, located in the hormone binding domain, and more precisely in the structural domain E from the carboxiterminal region of the protein, corresponding to the functional domain AF-2 (ligand-dependent transactivation). This region is related to the receptor dimerization, chaperone binding and recruitment of coregulators. Searching for SNP function prediction in the PUPASUITE and FASTSNP websites, we found that rs1801132 represents a target for the exonic splicing enhancers sc35 and sf2 (arginine serine-rich splicing factors) that interact with small nucleolar RNA and are required for the first step in the splicing reaction and spliceosome assembly. The thymus-specific exon ET of the c-myb protooncogene is encoded on the antisense strand of the sc34 gene. According to FASTSNP prediction report, the C allele of rs1801132 disrupts the binding site for GATA-1 and GATA-2 transcription factor.

For this SNP, our results showed a significant difference between the two control groups, suggesting that the C allele could be more frequent in women. In the literature, this SNP was investigated especially in relationship with breast cancer but the results were not convergent: some studies found the G allele protective [47] while other studies found increased risk with G allele [49–51] and another found no risk [52, 53] or found a risk only when included this polymorphisms in haplotypes [41, 54, 55]. In our study, we found the minor allele G protective for the total number of cancer cases.

Rs2228480 (G/A) (594Thr) is a silent polymorphism located in exon 8 of ESR1, within the F structural domain, respectively the functional domain TAF-2 (ligand-dependent transactivation). The functionality of this SNP is not known yet, but it seems to recruit coregulators. It was suggested that the C-terminal amino acids of ERalpha (the F domain) are critical for attenuation of E2 induced receptor dimerization and transcriptional activity; the F-domain mutants showed increases in dimerization. They also observed enhanced interaction of F domain mutants with p160 family coactivator SRC1 [56]. Accessing PUPASUITE website we observed that the polymorphic allele (A) disrupts the binding sites for the SR proteins SC35, SF2 and SRp40 (nucleolar, serine-rich protein). Overexpression of SRp40 was found during mouse mammary tumorigenesis; aberrant increases in SC35 have been associated with the cancer phenotype and SF2/ASF has been recently identified as a proto-oncogene [57–59].

In our cases of breast cancer, the heterozygous frequency was significantly higher than in controls. This SNP was in linkage disequilibrium with rs3798577 and we associated the haplotype C-G (f_{21}=0.448) with the risk of breast cancer, but the difference was not statistically significant (p=0.06). The previous studies associated the minor allele A of rs2228480 with an increased risk of breast cancer because of early exposure to estrogen (early onset of menarche) [48, 50, 60].

rs3798577(T/C) polymorphism is located in the 3'-UTR of ESR1. Although its functionality is not yet known, taken in account that 3'-UTR region is associated with the preferred target for microRNAs and splicing factors, it seems to modulate the ERα expression. Using PUPASUITE or FASTSNP websites, we were not able to find modulating factors that could include in their target this polymorphism.

Rs2228480 and rs3798577 were associated with survival and risk to develop distant metastasis (risk alleles A and C); G allele for rs2228480 is associated with an increased risk for relapse [46]. In accordance with the fore-mentioned publication, in our breast cancer cases, the C allele was strongly associated with the risk of breast cancer (p= 0.0007). We observed also that the strength of linkage disequilibrium between the two SNPs in the 3' region of ESR1 (rs2228480 and rs3798577) is low (D`=0.471, p= 0.03), despite them being separated by just over 1 kb.

In conclusion, our findings suggest that ESR1 polymorphisms may have a distinct impact in cancers, the same allele being associated with the risk or contrarily being protective depending upon the malignancy, suggesting different pathogenic implication of ER-signaling in the development of these malignancies. Further genotyping will establish whether these findings remain significant in larger cohorts and will allow further individualization of the clinical management regarding the surveillance program.

Acknowledgements This paper was supported by the Romanian grant no. 98/2007 PNII/CAPACITATI. We declare that we have no conflict of interest.

References

1. Dunning AM, Healey CS, Pharoah PD et al (1999) A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 8:843–854
2. Pharoah PD, Antoniou AC, Easton DF, Ponder BA (2008) Polygenes, risk prediction, and targeted prevention of breast cancer. N Engl J Med 358:2796–2803
3. Ripperger T, Gadzicki MA, Schlegelberger B (2009) Breast cancer susceptibility: current knowledge and implications for genetic counseling. Eu J Human Genetics 17:722–731
4. Pharoah PD, Tyrer J, Dunning AM et al (2007) Association between common variation in 120 candidate genes and breast cancer risk. PLoS Genet 3:e42
5. Gold B, Kirchhoff T, Stefanov S et al (2008) Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. PNAS 105:4340–4345
6. Easton DF, Pooley KA, Dunning AM et al (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447:1087–1093
7. Baynes C, Healey CS, Pooley CA et al (2007) Common variants in the ATM, BRCA1, BRCA2, CHEK2 and TP53 cancer susceptibility genes are unlike to increase breast cancer risk. Breast Cancer Res 9:R27
8. Gennari L, Merlotti D, De Paola V et al (2005) Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. Am J Epidemiol 161:307–320
9. Deroo BJ, Korach KS (2006) Estrogen receptors and human disease. J Clin Invest 116:561–570
10. Breivik RA, Lothe GI, Meling TO et al (1997) Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. Int J Cancer 74:664–667
11. Martineti V, Silvestri S, Tonelli F, Brandi ML (2008) Control of colon cancer development and progression by selected estrogen receptor modulators. Expert Rev Endocrinol Metab 3:503–511
12. Foley EF, Jazaeri AA, Shupnik MA et al (2000) Selective loss of estrogen receptor beta in malignant human colon. Cancer Res 60:245–248
13. Kouzmenko AP, Takeyama K, Kawasaki Y et al (2008) Ligand-dependent interaction between estrogen receptor alpha and adenomatous polyposis coli. Genes To Cells 13:723–730
14. Slattery ML, Sweeney C, Murtaugh M et al (2005) Associations between ERalpha, ERbeta, and AR genotypes and colon and rectal cancer. Cancer Epidemiol Biomarkers Prev 14:2936–2942
15. Liu W, Konduri SD, Bansal S et al (2006) Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. J Biol Chemistry 281:9837–9840
16. Teng J, Wang ZY, Jarrard DF, Bjorling DE (2008) Roles of estrogen receptor α and β in modulating urothelial cell proliferation. Endocr Relat Canc 15:351–364
17. Sun W, Zhong F, Zhi L et al (2009) Systematic-omics analysis of HBV-associated liver diseases. Cancer Lett 286:89–95
18. Deng G, Zhou G, Zhai Y et al (2004) Association of estrogen receptor Alpha polymorphisms with susceptibility to chronic hepatitis B virus infection. Hepatology 40:318–326
19. Zhai Y, Zhou G, Deng G et al (2006) Estrogen receptor alpha polymorphisms associated with susceptibility to hepatocellular carcinoma in hepatitis B virus carriers. Gastroenterology 130:2001–2009
20. Danel L, Cordier G, Revillard JP, Saez S (1982) Presence of estrogen binding sites and growth-stimulating effect of estradiol in the human myelogenous cell line HL60. Cancer Res 42:4701–4705
21. Hatfill SJ, Brusnicky J, Fester E (1991) Immunocytochemical identification of nuclear estrogen-receptors in human acute myeloid leukemia. Leuk Res 15:315–320
22. Skibola CF, Bracci PM, Halperin E et al (2008) Polymorphisms in the estrogen receptor 1 and vitamin C and matrix metalloproteinase gene families are associated with susceptibility to lymphoma. PLoS 3:e2816
23. Yuan HY, Chiou JJ, Tseng WH et al (2006) FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res 34(Web Server issue):W635–641
24. Conde L, Vaquerizas JM, Dopazo H, et al (2006) PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. Nucleic Acids Res 34(Web Server issue): W621-625 Available from http://bioinfo.cipf.es/pupasuite/
25. Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity, 86:248–249. http://genepop.curtin.edu.au/
26. Gaunt TR, Rodriguez S, Day INM (2007) Cubic exact solutions for the estimation of pair-wise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool 'CubeX'. BMC Bioinformatics 8:428–437. Available from http://www.oege.org/software/cubex/
27. International HapMap Consortium, Frazer KA, Ballinger DG, Cox DR et al (2007) A second generation human haplotype map of over 3.1 million SNPs. Nature 449:851–861
28. Nilsson M, Dahlmann I, Jiao H et al (2007) Impact of estrogen receptor gene polymorphisms and mRNA levels on obesity and lipolysis—a cohort study. BMC Medical Genetics 8:73–85
29. Zofková I, Zajícková K, Hill M (2002) The estrogen receptor alpha gene determines serum androstenedione levels in postmenopausal women. Steroids 67:815–819
30. Herrington DM, Howard TD, Hawkins GA (2002) Estrogen-receptor polymorphism and effects of estrogen replacement therapy on high-density lipoprotein cholesterol in women with coronary disease. N Engl J Med 346:967–974
31. Long JR, Xu H, Zhao LJ et al (2005) The estrogen receptor α gene is linked and/or associated with age of menarche in different ethnic groups. J Med Genet 42:796–800
32. Speer G, Cseh K, Winkler G (2001) Estrogen and vitamin D receptor (VDR) genotypes and the expression of ErbB-2 and EGF receptor in human rectal cancers. Eur J Cancer 37:1463–1468
33. Notarnicola M, Gristina R, Messa C (2001) Estrogen receptors and microsatellite instability in colorectal carcinoma patients. Cancer Lett 168:65–70
34. Kadiyska T, Yakulov T, Kaneva R et al (2007) Vitamin D and estrogen receptor gene polymorphisms and the risk of colorectal cancer in Bulgaria. Int J Colorectal Dis 22:395–400
35. Zuppan PJ (1989) Polymorphisms at the estrogen receptor locus and linkage relationships on chromosome 6q. Cytogenet Cell Genet 51:1116–1120

36. Yaich L, Dupont WD, Cavener DR, Parl FF (1992) Analysis of the PvuII restriction fragment-length polymorphism and exon structure of the estrogen receptor gene in breast cancer and peripheral blood. Cancer Res 52:77–83
37. Jakimiuk AJ, Nowicka M, Bogusiewicz M et al (2007) Prevalence of estrogen receptor α *Pvu*II and *Xba*I polymorphism in population of Polish postmenopausal women. Folia Histochem et cytobiol 45:331–338
38. Boyapati SM, Shu XO, Ruan ZX et al (2005) Polymorphisms in ER-α gene interact with estrogen receptor status in breast cancer survival. Clin Cancer Res 11:1093–1098
39. Moret-Onland CN, van Gils CH, Roest M et al (2005) The estrogen receptor α gene and breast cancer risk. Canc Causes Contr 16:1195–1202
40. Gonzalez-Zuloeta Ladd AM, Vasquez AA, Rivadeneira F et al (2008) Estrogen receptor α polymorphisms and postmenopausal breast cancer risk. Breast Cancer Res Treat 107:415–419
41. Wedren S, Lovmar L, Humphreys K et al (2004) Estrogen receptor α gene haplotype and postmenopausal breast cancer risk: a case control study. Breast Cancer Res 6:R437–R449
42. Anderson TI, Heimdal KR, Skrede M et al (2004) Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum Genet 94:665–670
43. Shin A, Kang D, Nishio H et al (2003) Estrogen receptor alpha gene polymorphisms and breast cancer risk. Breast Cancer Res Treat 80:127–131
44. Cai Q, Shu XO, Jin F et al (2003) Genetic polymorphisms in the estrogen receptor alpha gene and risk of breast cancer: results from the Shanghai breast cancer study. Cancer Epidemiol Biomarkers Prev 12:853–859
45. Tanaka Y, Sasaki M, Kaneuchi M et al (2003) Estrogen receptor alpha polymorphisms and renal cell carcinoma-a possible risk. Mol Cell Endocrinol 202:109–116
46. Tapper W, Hammond V, Gerty S, Ennis S, Simmonds P, Collins A, the Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) Steering Group and Diana Eccles (2008) The influence of genetic variation in 30 selected genes on the clinical characteristics of early onset breast cancer. Breast Cancer Res 10:R108–R118
47. Einarsdottir K, Darabi H, Li Y et al (2008) ESR1 and EGF genetic variation in relation to breast cancer risk and survival. Breast Cancer Res 10:R15–R24
48. Hsiao WC, Young KC, Lin SL, Lin PW (2004) Estrogen receptor–α polymorphism in Taiwanese clinical breast cancer population: a case-control study. Breast Cancer Res 6:180–186
49. Iwase H, Kobayashi S (1997) Alterations and polymorphisms of the estrogen receptor gene in breast cancer. Breast Cancer 4:57–66
50. Roodi N, Bailey LR, Kao WY et al (1995) Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst 87:446–451
51. Vasconcelos A, Medeiros R, Veiga I et al (2002) Analysis of estrogen receptor polymorphism in codon 325 by PCR-SSCP in breast cancer: association with lymph node metastasis. Breast J 8:226–229
52. Southey MC, Batten LE, McCredie MR et al (1998) Estrogen receptor polymorphism at codon 325 and risk of breast cancer in women before age forty. J Nat Cancer Inst 90:532–536
53. Gallichio L, Berndt S, McSorley MA et al (2006) Polymorphisms in estrogen-metabolizing and estrogen receptor genes and the risk of developing breast cancer among a cohort of women with benign breast disease. BMC Cancer 6:173–184
54. Gold B, Kalush F, Bergeron J et al (2004) Estrogen receptor genotypes and haplotypes associated with breast cancer risk. Cancer Res 64:8891–8900
55. Wang J, Higuchi R, Modugno F et al (2007) Estrogen receptor alpha haplotypes and breast cancer risk in older Caucasian women. Breast Cancer Res and Treat 106:273–280
56. Yang J, Singleton DW, Shaughnessy EA, Khan SA (2008) The F-domain of estrogen receptor-alpha inhibits ligand induced receptor dimerization. Mol Cell Endocrinol 295:94–100
57. Venables JP (2004) Aberrant and alternative splicing in cancer. Cancer Res 64:7647–7654
58. Lin S, Coutinho-Mansfield G, Wang D et al (2008) The splicing factor SC35 has an active role in transcriptional elongation. Nat Struct&Mol Biol 15:819–826
59. Shi J, Hu Z, Pabon K, Scotto KW (2008) Caffeine regulates alternative splicing in a subset of cancer-associated genes: a role for SC35. Mol Cell Biol 28:883–895
60. Long JR, Zhao LJ, Liu PY et al (2004) Pattern of linkage disequilibrium and haplotype distribution in disease candidate genes. BMC Genetics 5:11–18