Multiple Single Nucleotide Polymorphisms on Human Chromosome 19q13.2–3 Associate with Risk of Basal Cell Carcinoma[1]

Jiaoyang Yin, Eszter Rockenbauer, Mohammad Hedayati, Nicklas Raun Jacobsen, Ulla Vogel, Lawrence Grossman, Lars Bolund, and Bjørn A. Nexø[2]

Institute of Human Genetics, University of Aarhus, DK-8000 Aarhus C, Denmark [J. Y., E. R., L. B., B. A. N.]; Department of Medical Genetics, Shenyang Medical College, Shenyang 110031, Liaoning, People's Republic of China [J. Y.]; Department of Biochemistry, Johns Hopkins School of Public Health, Baltimore, Maryland 21205 [M. H., L. G.]; and Institute of Occupational Health, Lersø Parkalle 105, DK-2100 Copenhagen O, Denmark [N. R. J., U. V.]

Abstract

In this paper, we present evidence that alleles of several polymorphisms in the chromosomal region 19q13.2–3, encompassing the genes *RAI* and *XPD*, are associated with occurrence of basal cell carcinoma in Caucasian Americans. The association of one of these, *RAI*-intron1, is sufficiently strong to make mass significance unlikely ($P = 0.004$, χ^2). We interpret our combined data to indicate that a specific haplotype partly defined by the alleles of three single nucleotide polymorphisms, *RAI* intron1^G, *RAI* exon6^T, and *XPD* exon 6^C, is associated with a protective gene variant in a region spanning from *XPD* to *ERCC1*.

Introduction

SNPs[3] are the plankton of genetics. Individually they are tiny, 1-base differences in a 3-gigabase human genome, but by their multitude they constitute a rich source for genetic research. In the wake of the Human Genome Project, myriads of SNPs have surfaced, and it is now estimated that a SNP occurs approximately every 1000 bases. So no matter which part of the genome you are interested in, there are likely to be multiple SNPs located in the vicinity.

SNPs often effectively dichotomize human populations, making them useful for tracking genes of importance to disease in outbred populations by linkage disequilibrium. Specifically, typing of SNPs may facilitate the locating of regions of previously unknown importance for cancer risk. Thus, we have previously reported that polymorphisms in the gene *XPD* seemed associated with the occurrence of BCC (1). This finding has later been corroborated by the association of polymorphisms in *XPD* with BCC in a different population (2) and by the association of markers in the same gene with three other cancers, malignant melanoma (3), glioma (4), and lung cancer (5).

In this paper, we present evidence that alleles of several other polymorphisms in the chromosomal region 19q13.2–3, encompassing the genes *RAI* and *XPD*, are associated with occurrence of BCC. We are therefore convinced that a chromosomal variation influencing the risk of getting BCC and possibly other cancers must be located in this region, and we see the present paper as a first step toward identifying this variation.

Of the genes that we have investigated, *XPD*, *ERCC1*, and *LIG1* relate to DNA repair and are probably directly involved in preventing cancer. *FOSB*, which is a homologue of an oncogene, and *GLTSCR1*, which may be a tumor suppressor gene, might well also be involved in carcinogenesis or its prevention. For *RAI*, which seems to be involved in control of transcription, one might construct a relation to cancer prevention, but no experimental data to that effect are available. The remaining genes are unlikely to play a direct role in cancer. They were chosen simply because they were located in the chromosomal region of interest.

Materials and Methods

Study Groups. The groups of Caucasian Americans with and without BCC have been described previously (2, 6). DNAs for the analyses were purified from frozen lymphocytes obtained from blood.

Determination of Polymorphisms by LightCycler. Genotypes of polymorphisms in *CKM* exon 8 (position 20076, rs#4884) and *RAI* intron 1 (position 875, rs#1970764), *ERCC1* exon 4 (position 19007; Ref. 7), *FOSB* exon 4 (position 34621, rs#1049698), *SLC1A5* exon 8 (position 60620, rs#1060043), *LIG1* exon 6 (position 111, rs#20580), and *GLTSCR1* exon 1 (position 20775, rs#1035938) were detected using LightCycler (Roche Molecular Biochemicals, Mannheim, Germany; Ref. 8). The positions refer to the following accession numbers in GenBank: *RAI*, L47234; *CKM*, AC005781; *ERCC1*, M63796; *FOSB*, M89651; *SLC1A5*, AC008622; *LIG1*, L27710; and *GLTSCR1*; AC010519. The rs numbers refer to National Center for Biotechnology Information's database over SNPs, dbSNP. PCR was performed by rapid cycling in a reaction volume of 20 μl with 0.5 μM each primer, 0.045 μM anchor and sensor probe, 3.5 mM $MgCl_2$, 7 ng of genomic DNA, and 2 μl of LightCycler DNA Master Hybridization probe buffer (Roche Molecular Biochemicals, catalogue number 2158 825). This buffer contains Taq DNA polymerase, dNTP mix, and 10 mM $MgCl_2$. Table 1 shows the design of primers and fluorogenic probes for LightCycler. Some of the primers were copied from Ref. 8. Hobolth DNA (Hillerød, Denmark) and TIB-Molbiol (Berlin, Germany) synthesized the primers and probes, respectively. In

Received 9/21/01; revised 6/25/02; accepted 7/3/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by The Karen Elise Jensen Foundation, the Danish Cancer Society (Grant 9810028), the Danish Medical Research Council (Grant 9600259), the Danish SUE program (Grant 9800647-67), and the Novo Nordisk Foundation.
[2] To whom requests for reprints should be addressed, at Institute of Human Genetics, Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark. Phone: 45-8942-1686; E-mail: nexo@humgen.au.dk.
[3] The abbreviations used are: SNP, single nucleotide polymorphism; BCC, basal cell carcinoma; dNTP, deoxynucleotide triphosphate; SAP, shrimp alkaline phosphatase.

Table 1 Design of primers and fluorogenic probes for LightCycler

RAI intron 1
Forward primer: 5′-TGGCTAACACGGTGAAACC
Reverse primer: 5′-GGAATCCAAAGATTCTATGATGG
Anchor probe: 5′-GGGAGGCGGAGCTTGCAGTGA-fluorescein
Sensor probe: 5′-LCRed 640-CTGAGATCGCACCACTGCAC-p
CKM exon 8
Forward primer: 5′-TTGAAACTGGAACTCTGAGAAGG
Reverse primer: 5′-TGGTGGATGGTGTGAAGCA
Anchor probe: 5′-LCRed 640-CCTTTCTCCAACTTCTTCTCCATTTCC-ACC-p
Sensor probe: 5′-GGGGATCATGTCGTCAATGGACT-fluorescein
ERCC1 exon 4
Forward primer: 5′-AGGACCACAGGACACGCAGA-3′
Reverse primer: 5′-CATAGAACAGTCCAGAACAC-3′
Anchor probe: 5′-LCRed640-TGGCGACGTAATTCCCGACTATG-TGCTG p-3′
Sensor probe: 5′-CGCAACGTGCCCTGGGAAT-fluorescein
FOSB exon 4
Forward primer: 5′-AGGCTCAACAAGGAAAAATGC
Reverse primer: 5′-GCTAGACAGTCAAGGAGGGACG
Anchor probe: 5′-LCRed 640-AAAGGGTGGGTGTGGGAGACATTGG-p
Sensor probe: 5′-AAACCAACCTAGGCACCCCAAA-fluorescein
SLC1A5 exon 8
Forward primer: 5′-CAGTGTCCAAAGAGCACC
Reverse primer: 5′-CTACCCCTTTAGCGACC
Anchor probe: 5′-LCRed 640-TCCTGCCCCCAGAGCGTCACC-p
Sensor probe: 5′-GTACGGTCCACATAATTTTGGAGGA-fluorescein
LIG1 exon 6
Forward primer: 5′-ATGCCCTGTAGGTTCAATGG
Reverse primer: 5′-TGGAGGTCTTTAGGGGCTTG
Anchor probe: 5′-GGCTGGTCCCCGTCTTCTCCTTCC-fluorescein
Sensor probe: 5′-LCRed 640-TCTCTGTTGCCACTTCAGCCTC-p
GLTSCR1 exon 1
Forward primer: 5′-CGACGAACTTCTCTGAAGCGAA
Reverse primer: 5′-AGCGACACGGGCATCTGG
Anchor probe: 5′-ATGAGCGTCCACCTCCTGAACC-fluorescein
Sensor probe: 5′-LCRed 640-AGGCAGCAGCATCGTCATCCCC-p

Table 2 Design of primers and SNaPshot primers for sequenator

XRCC1 exon 7
Forward primer: 5′-GTCCCATAGATAGGAGTGAAAG
Reverse primer: 5′-CCCTAGGACACAGGAGCACA
SNaPshot primer: 5′-TGCATAGCTAGGTCCTGC
XRCC1 exon 17
Forward primer: 5′-GCCAAGCAGAAGAGACAAA
Reverse primer: 5′-GAGTGGCTGGGGAGTAGGA
SNaPshot primer: 5′-AACTGACRAAACTAGCTCTATGGGGTGGTGC-CGCA
RAI exon 6
Forward primer: 5′-CCTACCACCATCATCACATCC
Reverse primer: 5′-GCCTTGCCAAAAATCATAACC
SNaPshot primer: 5′-CCTCTCCCCAATTAAGTGCCTTCACACAGC
XPD intron 4
Forward primer: 5′-CGCAAAAACTTGTGTATTCACC
Reverse primer: 5′-CCCATTTTTATCATCAGCAACC
SNaPshot primer: 5′-CTGGCTCTGAAACTTACTAGCCC

Table 3 Design of primers and probes for Taqman

XRCC1 exon 10
Forward primer: 5′-GCT-GGA-CTG-TCA-CCG-CAT-G
Reverse primer: 5′-GGA-GCA-GGG-TTG-GCG-TG
Probe (A): 5′FAM[a]-TGC-CCT-CCC-**A**GA-GGT-AAG-GCC-T-TAMRA[a]
Probe (G): 5′VIC[a]-CCC-TCC-C**G**G-AGG-TAA-GGC-CTC-TAMRA[a]

[a] Tradenames of Applied Biosystems the bold underlined letter represents the polymorphic position.

some cases, the reaction mixture also contained 5% DMSO. The temperature cycling consisted of denaturation at 95°C for 2 s, followed by 46 cycles consisting of 2 s at 95°C, 10 s at 57°C, and 30 s at 72°C. The last annealing period at 72°C was extended to 120 s. The melting profile was determined by a temperature ramp from 50°C to 95°C with a rate of 0.1 degree/s. For *RAI* intron 1, we ran the melting profile three times and used the last curve.

Determination of Polymorphisms by Sequenator. The polymorphisms in *XRCC1* exon 7 (position 26651; Ref. 7), *XRCC1* exon 17 (position 36189; Ref. 7), *RAI* exon 6 (position 8786, rs#6966), and *XPD* intron 4 (position 19244, rs#1618536) were typed simultaneously on a ABI Prism 310 sequenator (Applied Biosystems, Foster City, CA) using the SNaPshot technique (9). The position numbers refer to the following accession numbers in GenBank: *XRCC1*, L34079; *RAI*, L47234; and *XPD*, L47234. The relevant primers are listed in Table 2. The PCR reaction consisted of 1 μl of purified genomic DNA, 1 pmol of each primer (DNA Technology, Aarhus, Denmark), 12.5 nmol of each dNTP (Bioline, London, United Kingdom), 100 nmol of $MgCl_2$ (Bioline), and 0.15 μl of BIOTAQ DNA polymerase (Bioline) in a total volume of 20 μl of water. The program consisted of 4 min at 96°C, followed by 25 cycles of 96°C for 30 s, 60°C for 30 s, and 72°C for 60 s. The last cycle was followed by 72°C for 6 min. The primers and dNTPs were removed in reactions containing 2 units of SAP (Roche Molecular Biochemicals), 2 units of exonuclease I (New England Biolabs, Beverly, MA), and 9 μl of PCR reaction in a total volume of 14 μl of water. The reactions were incubated at 37°C for 60 min and 72°C for 15 min. The SNaPshot reactions contained 1 μl of SNaPshot Ready Reaction Mix (Applied Biosystems), 0.5 μl of each of the SNaPshot primers (XRCC-e7-ss1, 4 pmol/μl; XPD-i5-cp1, 0.5 pmol/μl; RAI-e7-cp1, 1 pmol/μl; XRCC-e17-ss1, 2 pmol/μl), 2 μl of the purified PCR product, and 1.5 μl of buffer [200 mM Tris-HCl and 5 mM $MgCl_2$ (pH 9.0)]. The reactions were cycled 25 times: 96°C for 10 s, 50°C for 5 s, 60°C for 30 s. The primers and dNTPs were removed in a reaction containing 1 unit of SAP, 0.8 μl of 10× SAP buffer, and 5 μl of SNaPshot reaction in a total volume of 8 μl of water. Two μl of purified product were added to 10 μl of concentrated deionized formamide (Amresco), incubated for 5 min at 95°C, and analyzed on the sequenator. The two markers in *XRCC1*, in exon 7 and exon 17, could not be reliably scored, and thus we excluded these.

Determination of Polymorphisms by Real-Time PCR Using Taqman Probes. The polymorphism in *XRCC1* exon 10 (position 28152 - A399G, Ref. 7) was analyzed using the ABI Prism 7700 sequence detection system (Applied Biosystems). The position refers to GenBank accession number L34079. PCR primers and Taqman probes (Table 3) were designed using Primer Express v 1.0 (Applied Biosystems). TAG-Copenhagen Aps (Tagc.com, Copenhagen, Denmark) synthesized the primers, and Applied Biosystems synthesized the fluorescent Taqman probes. The reactions were performed in MicroAmp optical tubes sealed with MicroAmp optical caps (Applied Biosystems) containing a 10-μl reaction volume (1× Taqman buffer A; 2.5 mM $MgCl_2$; 200 μM each of dATP, dCTP, and dGTP; 400 μM dUTP; 800 nM each primer; 200 nM each probe; 0.01 unit/μl AmpErase UNG; and 0.025 unit/μl AmpliTaq Gold Polymerase). Thermal cycler conditions were as follows. Tubes were incubated at 50°C for 2 min followed by 10 min at

Table 4 Genotype occurrences of various SNPs among BCC cases and controls

SNP	Genotype	No. of controls	No. of cases (total)	No. of cases (<50 yr at onset)	*P* (total)[a]	*P* (<50 yr at onset)[a]
XRCC1 exon 10	AA	9	9	6	0.83	0.80
	AG	46	25	15		
	GG	42	29	18		
CKM exon 8	CC	57	33	23	0.52	0.93
	CT	36	24	13		
	TT	12	9	5		
XPD intron 4	AA	20	13	9	0.35	0.205
	AG	38	31	19		
	GG	39	14	7		
RAI exon 6	AA	60	46	28	0.059	0.019
	AT	28	10	6		
	TT	8	2	1		
RAI intron 1	AA	44	44	31	0.0042	0.00014
	AG	32	9	2		
	GG	3	1	0		
ERCC1 exon 4	AA	51	26	15	0.25	0.29
	AG	38	25	17		
	GG	18	14	8		
FOSB exon 4	CC	2	1	0	0.49	0.86
	CT	26	13	11		
	TT	88	57	34		
SLC1A5 exon 8	CC	80	55	32	0.40	0.069
	CT	11	11	10		
	TT	1	1	1		
GLTSCR1 exon 1	CC	55	34	23	0.76	0.39
	CT	32	22	13		
	TT	8	3	1		
LIG1 exon 6	AA	23	8	6	0.105	0.52
	AC	52	33	22		
	CC	30	24	12		

[a] Calculated from the allele occurrences using the χ^2 test.

95°C. The incubation was succeeded by 45 cycles of 95°C for 15 s and 64°C for 1 min. One sample of each genotype from a different cohort (1) previously analyzed for *XRCC1* exon 10 (results not published) using restriction enzyme analysis (10) was used as reference sample.

Statistical Methods. Data recording and tabulations as well as ANOVA analysis were performed with the program SPSS (SPSS, Chicago, IL). Fisher's exact test was performed online.[4] Bonferroni adjustment was performed online.[5] A global *P* was calculated from the individual levels of significance (p_i) using the formula:

$$\chi^2(P, 2n) = -2 \times \Sigma(\ln(p_i)) \quad (1)$$

where ln is the natural logarithm, *n* is the number of tests, and 2*n* is the degrees of freedom for χ^2. The χ^2-test was performed using Excel (Microsoft, Redmond, WA). The same program was used to calculate the odds ratios and related parameters. We used the program Arlequin,[6] which simulates the genotype data using Markov chains, to calculate the haplotype frequencies and the *P*s for linkage disequilibrium. The haplotype frequencies were then converted to Lewontin's normalized linkage disequilibrium D' using Excel and the formulas given online.[7]

Results

We have investigated the occurrence of the alleles of 10 new SNPs on chromosome 19q13.2–3 among Caucasian Americans with or without BCC. There is no scientific reason why we have used different techniques for typing different polymorphisms. The choices simply reflect which equipment was available to us at the times. Table 4 summarizes the results. Most SNPs showed no association with disease, but two markers (namely, *RAI* intron 1 and *RAI* exon 6) around the gene *RAI*, which is immediately 5′ to *XPD*, showed associations with BCC. In particular, the association of the *RAI* intron 1^A allele with cancer was fairly strong ($P = 0.004$; two-sided).

When we focused on those persons with early BCC (onset before 50 years of age), the difference in the distribution of the marker *RAI* intron 1 became even more pronounced ($P = 0.0002$; two-sided). As this would indicate, the average onset of BCC was later among cases carrying at least one *RAI* intron 1^G allele than among those homozygous for *RAI* intron 1^A [51.9 years ($n = 10$) *versus* 45.1 years ($n = 43$); $P = 0.022$; two-sided]. The heterozygotes also seemed to have fewer and later cases than expected. A similar analysis indicated that *RAI* exon 6^T also was associated with reduced risk of BCC. Not surprisingly, the two markers were in linkage disequilibrium, and the protective alleles were correlated.

Table 5 lists the frequencies of the rare alleles in controls and in all cases as well as in young cases (<50 years at onset). It is quite clear that the *RAI* intron 1^G allele is underrepresented in the cases, and there are suggestions that several of the neighboring SNP variants also differ in frequency between cases and controls.

Calculations of odds ratios with confidence intervals and *P*s for comparisons of BCC among those with two *RAI* intron 1^A *versus* those with at least one *RAI* intron 1^G are shown in Table 6 for both all BCC cases and early-onset BCC. Clearly,

[4] http://www.stat.ncsu.edu/~berger/tables.html.
[5] http://home.clara.net/sisa/bonfer.htm.
[6] http://lgb.unige.ch/arlequin/.
[7] http://www.fmv.ulg.ac.be/genmol/Guelph/Linkage%20disequilibrium.htm.

Table 5 Frequencies of the rare alleles of the SNPs in cases and controls

SNP	Allele	Controls	Cases (total)	Cases (<50 yrs)
XRCC1 exon 10	A	0.33	0.34	0.39
CKM exon 8	T	0.29	0.32	0.28
XPD intron 4	A	0.40	0.49	0.53
RAI exon 6	T	0.23	0.12	0.11
RAI intron 1	G	0.23	0.10	0.03
ERCC1 exon 4	G	0.35	0.41	0.41
FOSB exon 4	C	0.13	0.11	0.12
SLC1A5 exon 8	T	0.07	0.10	0.14
GLTSCR1 exon 1	T	0.25	0.24	0.20
LIG1 exon 6	A	0.47	0.35	0.43

Table 6 Odds ratios for the SNP *RAI* intron 1 and total and early BCC

	AA	AG & GG
Total BCC		
Controls	44	35
Cases	44	10
Odds ratio	1	0.286
Confidence interval		0.13–0.65
P^a		0.0027
BCC before 50 yr of age		
Controls	44	35
Cases	31	2
Odds ratio	1	0.081
Confidence interval		0.018–0.36
P^a		<0.00009

[a] Fisher's exact test, two-sided.

Table 7 Odds ratios for the SNP *RAI* intron 1 and BCC subdivided according to family history for BCC

	AA	AG & GG
Negative family history		
Controls	39	29
Cases	30	7
Odds ratio	1	0.313
Confidence interval		0.12–0.81
P^a		0.015
Positive family history		
Controls	5	6
Cases	14	3
Odds ratio	1	0.179
Confidence interval		0.032–0.99
P^a		0.057

[a] Fisher's exact test, two-sided.

the odds ratios were less than 1, in keeping with the idea that the *RAI* intron 1^G allele was protective. Similar calculations, where the cases and controls were subdivided according to their report of BCC occurring in their family, are shown in Table 7. The association of the SNP with the risk of BCC seemed to be present irrespective of family history.

We have made similar calculations for SNP *RAI* exon 6. Here the T allele was marginally associated with reduced risk of BCC (results not shown).

The previously reported values for DNA repair capacity (6) appeared linearly related to the marker *RAI* exon 6 in those persons without a family history for BCC [7.5 ± 2.2 (n = 110), 8.4 ± 2.8 (n = 37), and 9.4 ± 1.8 (n = 2) for AA, AT, and TT, respectively; P = 0.035, ANOVA]. Among those persons with a family history, there also was a relation to *RAI* exon 6, but it was nonlinear [8.1 ± 1.8 (n = 24), 6.4 ± 2.0 (n = 7), and 8.8 ± 0.9 (n = 2) for AA, AT, and TT, respectively; P = 0.043, ANOVA].

Table 8 lists the normalized linkage disequilibria between the markers in cases and controls together (this investigation and Ref. 2) and also lists the *P*s for the linkage disequilibria. Many of the markers were in strong linkage disequilibrium. Presumably, in reality, they are located close together on chromosome 19. We have organized the markers so that strong disequilibria correspond to short distances. The map corresponds to the current National Center for Biotechnology Information map of chromosome 19.

Discussion

In the present paper, we have searched for an association of BCC with 10 different SNPs on chromosome 19q13.2–3. The alleles of neighboring SNPs correlated moderately, and one or two of the SNPs were individually associated with disease.

Bonferroni adjustment is a formula that specifies how low the lowest of a series of *P*s must be to make the tests as a whole significant, *i.e.*, it is a way of correcting for mass significance when multiple tests are made. Bonferroni adjustment allows the different tests to be partly related and includes a way to correct for this by using the mean absolute correlation coefficient of the variables as a parameter. When we performed Bonferroni adjustment for the multiplicity of tests (in total, 10 tests) of variables with a mean absolute correlation coefficient of 0.14, the results indicated that any $P < 0.007$ would make the whole set significant at the level of 0.05. The P for an association of the marker *RAI* intron 1 with cancer was lower (P = 0.004), *i.e.*, this association was still significant after correction for the multiplicity. Leaving out the correction for the correlation among the variables did not change this conclusion. We have also calculated the global P from the 10 *P*s for associations between all markers and BCC using a formula (Ref. 1; see "Materials and Methods") that assumes no correlation between variables. The result was a global P of 0.045. Thus, either way, the result is globally significant. Moreover, using the formula (1) on early skin cancers further reduced this global P considerably (P = 0.004). Consequently, it is unlikely that our results were caused by mass significance; rather they indicate the presence of a genetic variation influencing the risk of BCC.

In a previous paper we suggested that such a causative variation influencing the risk of skin cancer should be found in the 5′ end of *XPD* or 5′ to the gene (2). In the present paper we show that two markers next to or inside the gene *RAI*, located immediately 5′ to *XPD*, are associated with risk of BCC. Thus, a total of three closely linked markers seem to confer resistance: *RAI* intron 1^G; *RAI* exon 6^T; and *XPD* exon 6^C. As one would expect the three alleles associated with protection tended to occur together. Presumably, the causative gene variation is located nearby. The data suggest fairly strongly that the etiological mutation resides in a block of linkage disequilibrium that encompasses at least *XPD* and *RAI*. However, deviations from monotonicity are known to occur in maps of linkage disequilibrium, so the boundaries must be viewed with caution (11). Neither of the polymorphisms in *RAI* changes protein coding. Also, we would like to point out that a slight ambiguity exists with respect to the positions of the markers *RAI* intron 1 and *RAI* exon 6. Previous maps of the region put the markers at different locations in the gene.

Our results are strong evidence that occurrence of BCC, the most common cancer among Caucasians, is genetically

Table 8 Normalized linkage disequilibrium D' and *P*s for linkage disequilibrium among markers on chromosome 19q13.2–3 among 129 to 176 Caucasian Americans[a]

	xr1e10	ckme8	xpde23	xpde10	xpde6	xpdi4	raie6	raii1	er1e4	fosbe4	slc1a5	glt1e1	lig1e6
xr1e10		−0.38											
Ckme8	*0.04*		*−0.52*	*0.37*	**−0.5**	*−0.44*							
Xpde23		*1E-04*		**0.7**	**0.72**	**0.77**	**−0.8**	*−0.63*	**0.31**	−0.32			
Xpde10		*0.003*	**1E-26**		**−0.85**	**−0.82**	*0.56*		**−0.26**				
Xpde6		**7E-07**	**1E-13**	**2E-20**		**0.91**	**1**	**1**	0.23	*−0.27*			
Xpdi4		*2E-04*	**1E-16**	**5E-21**	**0**		**0.93**	*0.84*	0.25	*−0.46*			
Raie6			**4E-06**	*0.002*	**2E-08**	**4E-07**		**0.93**	*−0.7*				
Raii1			*0.004*		**3E-08**	*1E-04*	**1E-21**						
er1e4			**2E-05**	**8E-05**	0.02	*0.009*	*0.002*			**−0.7**			
fosbe4			0.07		*0.004*	*0.002*			**1E-06**				
slc1a5													1
glt1e1											0.04		
lig1e6													

[a] Above the diagonal, D'-values; below the diagonal, *P*s; bold letters, $P < 10^{-4}$; italic letters, $P < 10^{-2}$; values corresponding to $P > 0.1$ have been left out.

nonrandom. This is not at issue with the fundamental concept that carcinogenesis is a stochastic process, but it does indicate that the parameters governing this process differ between persons. Ultimately, the treatment of the majority of the population as homogeneous *vis-à-vis* cancer may prove fallible.

The present data, which locate a gene influencing cancer occurrence to a certain region of chromosome 19q13.2–3, only relate to BCC. However, it is likely that whatever gene variation causes the differences also influences other forms of cancer. First, many protective mechanisms against cancer are rather general in nature, *e.g.*, DNA repair. Secondly, a number of studies of other cancer forms have also found associations of markers in this region with disease (3–5). In this context, it should be noted that others have described evidence of a glioma tumor suppressor gene in the same general region (12). We think this is a different gene because the present map of chromosome 19 puts it at a fair distance (13). Neither *XRCC1* nor *LIG1* seems to play a role in our study.

These investigations were started with the notion that variations in nucleotide excision repair genes could influence the risk of getting cancer, and it is still quite possible that a difference in the 5′ end of *XPD* (for instance, in a regulatory region) modulates repair. Alternatively, *RAI*, which is known to play a role in transcription (14), may be involved in regulation of genes for DNA repair.

We have reported previously that the mRNA levels of *XPD* and *ERCC1* in lymphocytes are closely correlated and also correlate with the DNA repair capacity in such cells (15). It is intriguing that the region described here as related to occurrence of skin cancer may span regulatory regions for both genes and that a genetic variation in this region is correlated to DNA repair capacity.

Definitive identification of the causative gene will likely require sequencing of the genomic region on disease-associated *versus* non-disease-associated chromosomes. We have embarked on this project.

Acknowledgments

We thank Thrine Schneidermann for expert technical assistance, Helle Binderup for help with the SNaPshot typing, and Dr. Olfert Landt (TIB-Molbiol) for advice on the design of probes for the LightCycler.

References

1. Dybdahl, M., Vogel, U., Frentz, G., Wallin, H., and Nexø, B. A. Polymorphisms in the DNA repair gene XPD: correlations with risk and age at onset of basal cell carcinoma. Cancer Epidemiol. Biomark. Prev., *8:* 77–81, 1999.

2. Vogel, U., Hedayati, M., Dybdahl, M., Grossman, L., and Nexø, B. A. Polymorphisms of the DNA repair gene XPD: correlations with risk of basal cell carcinoma revisited. Carcinogenesis (Lond.), *22:* 899–904, 2001.

3. Tomescu, D., Kavanagh, G., Ha, T., Campbell, H., and Melton, D. W. Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. Carcinogenesis (Lond.), *22:* 403–408, 2001.

4. Caggana, M., Kilgallen, J., Conroy, J. M., Wiencke, J. K., Kelsey, K. T., Miike, R., Chen, P., and Wrensch, M. R. Associations between ERCC2 polymorphisms and glioma. Cancer Epidemiol. Biomark. Prev., *10:* 355–360, 2001.

5. Butkiewicz, D., Rusin, M., Enewold, L., Shields, P. G., Chorazy, M., and Harris, C. C. Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis (Lond.), *22:* 593–597, 2001.

6. Wei, Q., Matanoski, G. M., Farmer, E. R., Hedayati, M. A., and Grossman, L. DNA repair and aging in basal cell carcinoma: a molecular epidemiology study. Proc. Natl. Acad. Sci USA, *90:* 1614–1618, 1994.

7. Shen, M. R., Jones, I. M., and Mohrenweiser, H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res., *58:* 604–608, 1998.

8. Wittwer, C. T., Ririe, K. M., Andrew, R. V., David, D. A., Gundry, R. A., and Balis, U. J. The LightCycler™: a microvolume multisample fluorimeter with rapid temperature control. Biotechniques, *22:* 176–181, 1997.

9. Lindblad-Toh, K., Winchester, E., Daly, M. J., Wang, D. G., Hirschhorn, J. N., Laviolette, J. P., Ardlie, K., Reich, D. E., Robinson, E., Sklar, P., Shah, N., Thomas, D., Fan, J. B., Gingeras, T., Warrington, J., Patil, N., Hudson, T. J., and Lander, E. S. Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. Nat. Genet., *24:* 381–386, 2000.

10. Lunn, R. M., Langlois, R. G., Hsieh, L. L., Thompson, C. L., and Bell, D. A. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res., *59:* 2557–2561, 1999.

11. Taillon-Miller, P., Bauer-Sardiña, I., Saccone, N. L., Putzel, J., Laitinen, T., Cao, A., Kere, J., Pilia, G., Rice, J. P., and Kwok, P-Y. Juxtaposed regions of extensive and minimal linkage disequilibrium in human Xq25 and Xq28. Nat. Genet., *25:* 324–328, 2000.

12. Rosenberg, J. E., Lisle, D. K., Burwick, J. A., Ueki, K., von Deimling, A., Mohrenweiser, H. W., and Louis, D. N. Refined deletion mapping of the chromosome 19q glioma tumor suppressor gene to the D19S412-STD interval. Oncogene, *13:* 2483–2485, 1996.

13. Smith, J. S., Tachibana, I., Pohl, U., Lee, H. K., Thanarajasingam, U., Portier, B. P., Ueki, K., Ramaswarmy, S., Billings, S. J., Mohrenweiser, H. W., Louis, D. N., and Jenkins, R. B. A transcript map of chromosome 19q-arm glioma tumor suppressor region. Genomics, *64:* 44–50, 2000.

14. Yang, J. P., Hori, M., Sanda, T., and Ohamoto, T. Identification of a novel inhibitor of nuclear factor-κB, RelA-associated inhibitor. J. Biol. Chem, *274:* 15662–15670, 1999.

15. Vogel, U., Dybdahl, M., Frentz, G., and Nexø, B. A. DNA repair capacity: inconsistency between effect of over-expression of five NER genes and the correlation to mRNA levels in primary lymphocytes. Mutat. Res., *461:* 197–210, 2000.

Multiple Single Nucleotide Polymorphisms on Human Chromosome 19q13.2–3 Associate with Risk of Basal Cell Carcinoma

Jiaoyang Yin, Eszter Rockenbauer, Mohammad Hedayati, et al.

Cancer Epidemiol Biomarkers Prev 2002;11:1449-1453.