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Segregation analysis of urothelial cell carcinoma

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ABSTRACT

A family history of urothelial cell carcinoma (UCC) confers an almost two-fold increased risk of developing UCC. It is unknown whether (part of) this aggregation of UCC has a Mendelian background. We performed complex segregation analyses on 1193 families ascertained through a proband with UCC of the bladder, ureter, renal pelvis or urethra, who were newly diagnosed between January 1, 1995 and December 31, 1997 and registered by two population-based cancer registries in the southeastern part of the Netherlands. Data were reported on 10 738 first-degree relatives by postal questionnaire; 101 of these relatives had UCC. All reported occurrences of UCC were verified (if possible) using medical records. Analyses were performed with the S.A.G.E. segregation package. Five restricted models (Mendelian dominant, Mendelian recessive, Mendelian co-dominant, 'no major gene' model and environmental model) were tested against the general unrestricted model. Sex and smoking status were incorporated as covariates. Strong evidence of Mendelian inheritance of UCC through a single major gene was not found in these 1 193 families. However, since none of the Mendelian models could be rejected, an inherited subtype of UCC cannot be excluded. A major gene may segregate in some families but this effect may have been masked in a background of high sporadic incidence. The 'no major gene' (or sporadic) model appeared to be the most parsimonious one to describe the occurrence of UCC in these families.

1. Introduction

Although urothelial cell carcinoma (UCC) is not known as a familial cancer, several case-reports and population-based studies have described familial clustering of UCC.[1] Many of the case-reports presented families with UCC patients diagnosed at a very early age, suggesting a genetic component. Only a few studies specifically addressed the issue of familial

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UCC. Kramer and colleagues[2] reported a (non-significant) two-fold increased risk for first-degree relatives of 319 male UCC patients from the state of New York vs. first-degree relatives of 319 male neighborhood controls. By contrast, a study from Iceland[3] reported only a slightly increased risk (not statistically significant) of UCC among relatives of UCC cases (observed-to-expected (O/E) ratio = 1.2). Unexpectedly, the O/E ratio was higher among second (O/E = 1.4) and third-degree (O/E = 1.3) relatives than among first-degree relatives (O/E = 1.0). We previously reported the first study with sufficient statistical power to detect familial aggregation of UCC.[4] Case families were ascertained through UCC patients (probands), who were registered within a 3-year period in two population-based cancer registries. Control families consisted of the families of the probands' partners. A random coefficient proportional hazard analysis yielded an age, sex and smoking adjusted Hazard Ratio (HR) of 1.8 (95% Confidence Interval (CI):1.3–2.7) of UCC among first-degree relatives of 1193 UCC patients compared to first-degree relatives of 853 controls. A clear clustering of tumours at other sites among the family members of UCC patients was not found.

Dong and Hemminki[5] used the Swedish Family Cancer Database to quantify the risk of cancer in more than 5.5 million offspring from more than 2 million nuclear families if one of the parents had cancer or if one or more of the siblings had cancer. The risk of bladder cancer was increased with a factor 1.5 (95%CI:1.1–2.0) if one of the parents had bladder cancer. If one of the siblings had bladder cancer, the risk was increased with a factor 3.3 (95%CI:1.7–5.8). In a twin study from Scandinavia, Lichtenstein [6] reported 5 concordant and 146 discordant pairs of bladder cancers among 7231 monozygotic male twin pairs. Among 13769 dizygotic twin pairs, 2 concordant vs. 253 discordant pairs were found. The concordance rate among monozygotic vs. dizygotic twins was 3 times higher, suggesting a genetic etiology.

Overall, the findings reported in the case-reports and population-based studies support the existence of a familial subtype of UCC that appears to be site-specific and not part of any known hereditary cancer syndrome. However, the question whether the familial aggregation can best be explained by environmental or genetic factors has not been addressed thoroughly. In this study complex segregation analysis was performed to test whether the pattern of UCC in families is consistent with genetic transmission. If an inherited subtype of UCC exists, gene mapping studies may be useful. It may also serve as a model for understanding genetic alterations underlying sporadic UCC tumourigenesis in general.

2. Patients and methods

The study base is defined as the population time (age) of all first-degree relatives of patients with UCC. From this study base, we selected as the study population, all 10738 first-degree relatives of the 1193 UCC patients identified in our study on familial aggregation of UCC.[4] These patients were newly diagnosed between January 1, 1995 and December 31, 1997 and identified through two population-based cancer registries managed by the Comprehensive Cancer Centres East and South in the Netherlands.

Inclusion of patients was independent of family history of UCC. Of the 1193 patients, 1081 were diagnosed with bladder cancer, 57 with cancer of the renal pelvis cancer, 45 with cancer of the ureter and 10 with cancer of the urethra (International Classification of Diseases for Oncology, first edition,[7] topography codes 188.1-9 and 189.1-3, and ICD-O, second edition,[8] morphology codes 8120 and 8130). All patients were Caucasian, younger than 75 years at diagnosis and they had to be capable of reading and understanding the Dutch language. Information on demographic factors, smoking habits, occupation, history of cancer, history of urinary tract diseases, history of chronic diseases and drug history was collected by postal questionnaire.

In the same questionnaire similar information was requested on first-degree relatives (demographic factors, smoking habits, longest held occupation, history of cancer and history of urinary tract diseases). In case the patient was not able to provide information, the partner (or next-of-kin) was contacted for information. All data on cancer of the urinary tract in relatives of the study participants were verified (if possible) with medical records, after written consent of the patients themselves or, in case of death, their partners or next-of-kin. Verification appeared to be impossible, however, for relatives with UCC who had died more than 15 years before the study. A more detailed description of the data collection has been reported previously.[4] This study was approved by the Institutional Review Boards of the Radboud University Nijmegen Medical Centre, the Comprehensive Cancer Centre East and Comprehensive Cancer Centre South.

3. Segregation analysis

Complex segregation analysis evaluates models of disease transmission by looking at family data. Both genetic and environmental factors may be influencing the observed pattern of disease occurrence in families. The genetic factors may be polygenic or Mendelian with any mode of inheritance or any mixture of these.

To determine whether a major gene is involved in UCC, susceptibility and to predict the pattern of inheritance of the hypothesized gene, maximum likelihood estimates for several parameters were obtained. The parameters estimated in complex segregation analysis are: 1) an underlying discrete risk trait (that may be present in double doses (AA), one dose (AB) or absent (BB)) that influences a given individual's age-dependent risk for disease (in genetic models, this trait represents a high-risk allele, whereas in non-genetic models, the trait is interpreted more generally as levels of exposure to an unmeasured major environmental risk factor); 2) transmission parameters which represent the probability that a parent transmits the risk trait to an offspring; and 3) the penetrance of the risk trait.

The computer performs a maximum likelihood analysis to find the combination of parameter values which gives the largest overall likelihood for the observed data. A general, non-restricted model is fitted to the data and will give the best fit. Five restricted models (Mendelian dominant, Mendelian recessive and Mendelian co-dominant, 'no major gene' and environmental model) will be tested against the general model. The Mendelian models assume a major locus with

two alleles that act either in a dominant, co-dominant or recessive fashion. The 'no major gene' model assumes that the baseline risk is not influenced by the risk trait (i.e. all persons have the same specific risk of UCC). The environmental model assumes that an individual's phenotype depends on his or her environmental exposures and is independent of the phenotype of the parents. The fit of the general unrestricted model was compared to that of each of the five models by the likelihood ratio test (LRT). Twice the difference in the models' ln-likelihood is asymptotically distributed as a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters estimated in the two models under the null hypothesis. We also compared the models using the Akaike's information criterion (AIC)[9] defined as AIC = $-2\ln(L) + 2$ (number of parameters estimated): the most parsimonious model is that with the lowest AIC value.

The statistical software package SAGE (Statistical Analysis for Genetic Epidemiology)[10] was used for the complex segregation analysis. More detailed information on the segregation analysis is given in Appendix 1.

4. Results

4.1. Population characteristics

The 1193 UCC probands had 10738 first-degree family members and 1092 partners. Among the 10738 first-degree relatives, 75 males and 26 females were reported to have UCC. Mean age at diagnosis was approximately 64 years, for both males and females. Eighty-nine probands (7.5%) had one first-degree relative diagnosed with UCC and 6 (0.5%) had two first-degree relatives diagnosed with UCC. In Table 1, the characteristics of probands, partners and first-degree relatives are shown. Disease status was unknown in 239 individuals (all partners). Age at diagnosis, age at time of questionnaire or age at death was missing for 802 first-degree relatives, 7.5% of the total cohort. Of these 802 subjects, 362 (45.1%) were female and 440 (54.9) were male. The geometric mean age for all relatives was 54.6 years (age at diagnosis or age at time of questionnaire / death in case of non-affected relatives).

4.2. Segregation analysis

The results of the segregation analyses, including sex and smoking as covariates, are presented in Table 2. All environmental, 'no major gene' and single gene models were not significantly different from the general, unrestricted model. Comparing the models using Akaike's criterion the 'no major gene' model had the lowest AIC value and was therefore the most parsimonious model.

When we tried to fit a more complicated, sex-dependent model (results not shown) the age-dependent parameters appeared to be similar for males and females. Therefore, we used the more simple models including sex as covariate.

5. Discussion

This study was carried out to investigate a possible Mendelian inheritance as explanation for the reported familial aggregation of UCC. All of the hypothesized models were not significantly different from the general, unrestricted model. Although the transmission parameters of the general model indicated a Mendelian inheritance, none of the Mendelian models gave a much better fit to the data than the environmental and 'no major gene' models. Based on Akaike's information criterion the 'no major gene' model was the most parsimonious one. This suggests that a major gene is not involved in the development of UCC. Because smoking is the main risk factor for bladder cancer, accounting for 30–50% of all bladder cancers, smoking was taken into account as a covariable in all models in order to minimize its influence. However, our data on smoking behavior of all family members is based on proxy data (i.e. all data of the family members is retrieved by the proband) and therefore, the adjustment for smoking may not have been perfect.

Because smoking behavior as well as dietary habits may be correlated among family members, it may be possible that these factors would be the underlying cause(s) for the observed clustering of bladder cancer in families. In that case, the analysis should have revealed the environmental model

Table 1 – Characteristics of the probands and their first-degree relatives

	Probands	1st-degree relatives of proband		
		Parents	Siblings	Children
N	1193	2386	5628	2724
Male / Female (%)	988 / 205 (17/83)	1193 / 1192 (50/50)	2774 / 2854 (49/51)	1404 / 1320 (51/49)
Smoking behavior				
Smokers (%)	1090 (91.4)	1165 (48.8)	2991 (53.1)	1321 (48.5)
Non-smokers (%)	103 (8.6)	1081 (45.3)	2023 (35.9)	1330 (48.8)
Unknown (%)	0	140 (5.9)	614 (10.9)	73 (2.7)
Affected				
Yes (%)	1193 (100)	54 (2.3)	46 (0.8)	1 (0.0)
Mean age at diagnosis (SD[a])	61.8 (9.5)	67.2 (10.3)	60.6 (9.6)	25.0 (–)
No (%)	0	2332 (97.7)	5582 (99.2)	2723 (100.0)
Mean age at questionnaire (SD[a])	–[b]	74.2[c] (13.1)	61.6 (12.3)	35.7 (7.4)

a SD = Standard Deviation.
b All probands are diagnosed with UCC.
c Mean age at questionnaire or mean age at death if deceased.

Table 2 – Results of the complex segregation analyses; the general unrestricted model is compared to five restricted models (Mendelian dominant, Mendelian recessive, Mendelian co-dominant, 'no major gene' and environmental model)

	Hypothesis					
	Mendelian			No major gene	Environmental $q_A = \tau_{AA} = \tau_{AB} = \tau_{BB}$	General
	Dominant	Recessive	Co-dominant			
q_A	0.004 (0.02)	0.141 (0.107)	0.005 (0.02)	[1.0]	0.084 (0.03)	0.124 (0.11)
τ_{AA}	[1.0]	[1.0]	[1.0]	–	$=q_A$	{1.0}
τ_{AB}	[0.5]	[0.5]	[0.5]	–	$=q_A$	0.456 (0.20)
τ_{BB}	[0.0]	[0.0]	[0.0]	–	$=q_A$	{0.0}
β_{AA}	−30.76 (5.89)	−32.69 (4.57)	−28.32 (7.59)	−32.48 (2.91)	−42.37 (10.50)	−32.68 (4.60)
β_{AB}	$=\beta_{AA}$	−35.86 (4.28)	−31.14 (5.82)	$=\beta_{AA}$	−48.51 (11.32)	−35.46 (4.58)
β_{BB}	−33.76 (4.19)	$=\beta_{AB}$	−33.97 (4.32)	$=\beta_{AA}$	−51.88 (12.29)	−36.48 (4.55)
α	0.128 (0.02)	0.137 (0.018)	0.129 (0.02)	0.124 (0.01)	0.201 (0.05)	0.137 (0.02)
γ	0.130 (0.06)	0.117 (0.047)	0.129 (0.06)	0.113 (0.04)	0.080 (0.02)	0.137 (0.08)
Smoking	0.649 (0.40)	0.666 (0.414)	0.644 (0.39)	0.573 (0.37)	0.830 (0.55)	0.686 (0.42)
Sex	1.376 (0.39)	1.476 (0.408)	1.385 (0.39)	1.341 (0.36)	2.260 (0.75)	1.467 (0.42)
−2lnL	1654.44	1654.13	1654.43	1656.73	1654.92	1653.56
AIC	1668.44	1668.13	1670.43	1666.73	1670.92	1675.56
Df	4	4	3	5	3	–
χ^2	0.88	0.57	0.87	3.17	1.26	–
P-value	0.93	0.97	0.83	0.79	0.74	–

All values between [ ] are fixed, all values between {} converge at the boundary.
#All parameters are clarified in detail in the appendix.
q_A is defined as the allele frequency of the high risk allele.
τ_{AA}, τ_{AB}, τ_{BB} are the transmission parameters which define the probability that a parent transmits the high risk allele to the offspring. In the no major gene model these factors were not included and in the environmental model these factors were set equal to the frequency of the allele frequency assuming complete homogeneity of environmental exposures across generations.
β_{AA}, β_{AB}, β_{BB} are the baseline parameters per type (types representing genotypes in the genetic models and in the non-genetic models, type can be interpreted as levels of exposure to an unmeasured major environmental risk factor that is not correlated among relatives).
α is the age coefficient.
γ is the susceptibility coefficient which is defined as the cumulative probability of developing UCC if one lives to age infinity.
Smoking (ever vs. never) and sex are included as covariates.
χ^2 is defined as (−2lnL) of the data under the hypothesis minus (−2lnL) of the data under the general model.
AIC is Akaike's information criteria defined as AIC = −2ln(L) + 2(number of parameters estimated).

as the most likely model, fitting the data equally good as the general unrestricted model.

In theory, the inclusion of families with a known cancer family syndrome, especially Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC) in which upper urinary tract UCC clusters, could have biased the results. However, none of the included families met the HNPCC criteria. Another possibility is that including all UCCs (bladder and upper urinary tract) in the analyses, distorted the segregation analysis. However, we think this is unlikely because less than 10% of the probands had an upper urinary tract UCC and only 5% of the affected family members were diagnosed with upper urinary tract UCC. Furthermore, none of the families with at least 2 affected family members had both individuals diagnosed with upper urinary tract UCC. Stratified analyses showed similar familial aggregation for probands with bladder UCC vs. upper urinary tract UCC (HR = 1.9 vs. 1.8, respectively).

In a study from Utah,[11] the risk ratio for bladder cancer among first-degree relatives of young probands (<60 years) increased from 1.5 to 5.1. Also, in our own study on familiality of UCC the HR of 1.8 (95%CI: 1.2–2.6) among first-degree relatives increased to 2.5 (95%CI:2.0–4.0) when only young probands were included. Stratified segregation analyses including only probands younger than the age of 60 were therefore considered but could not be performed because of limited power.

When interpreting the results of segregation analyses, some critical limitations of such analyses have to be considered. The S.A.G.E. package assumes that any major gene inheritance occurs through a single two-allele autosomal locus. In reality, the inheritance pattern may be more complex, making the identification of a specific model more difficult. Another limitation is that of statistical power. The effect of a rare major gene may remain masked, under the overwhelming number of 'sporadic' UCC cases. Although almost 1200 families were included in this study, lack of power may be an explanation for the findings, since none of the models examined could be rejected. The involvement of a genetic factor seems obvious considering the striking case-reports but the influence of this genetic factor cannot easily be detected by segregation analysis. The inclusion of more individuals, especially larger-sized families (i.e. inclusion of second-degree relatives) may improve power to detect genetic mechanisms underlying transmission of UCC. In this study, information was collected from second-degree relatives but not included in the analyses because the data were incomplete and difficult to validate.

Other methods have to be applied, in order to identify the involvement of a genetic factor. Linkage analyses may be performed when one or more extended families are identified with multiple relatives affected with UCC. Unfortunately, in this study such families were not found. An alternative possibility in the absence of extended families with multiple affected members is affected sib-pair gene mapping. For this, an international collaborative effort to collect sib-pairs will be necessary in order to reach sufficient power. In our hospital, we are presently conducting high-resolution array comparative genomic hybridization (CGH) analyses (with a resolution of approximately 100 kb) in families with three first-degree UCC patients in the hope to identify small genomic deletions, which may harbor UCC susceptibility genes.[12]

In 1996 a UCC family was reported by Schoenberg.[13] In this family, a male patient (diagnosed at the age of 27) had a mother who died from bladder cancer at the age of 65 years. Triggered by a history of several miscarriages in his wife and mother, karyotype analysis was performed yielding a constitutional balanced translocation t(5;20)(p15;q11). Detailed study of the break points has recently revealed a new bladder cancer gene at 20q11 (*CDC91L1*, encoding CDC91L1, also called phosphatidylinositol glycan class U (PIG-U)).[14] This gene has a role in the glycosylphosphatidylinositol (GPI anchoring pathway). Further research suggested that the gene is amplified and overexpressed in 1/3 of all bladder cancers (although this was not confirmed in an independent study of Schulz[15]). *CDC91L1* can therefore be considered as an oncogene. The translocation led to overexpression of the gene and probably to both bladder cancers in this pedigree. However, the exact translocation in this family should be regarded as an extremely rare phenomenon. The *PIG-U* gene should therefore not be considered candidate for the genetic cause of many patients with hereditary bladder cancer. For that tumour suppressor or DNA mismatch repair genes have yet to be discovered.

A highly penetrant gene which is known to increase the risk of bladder cancer is the *RB1* gene. Relatives of retinoblastoma patients (carriers of the mutated *RB1* gene) showed an increased risk of bladder cancer.[16–18] Also in a study from the U.K., survivors of hereditary retinoblastoma appear to have an increased risk of bladder cancer (O/E ratio = 26.3, 95%CI:8.5–61.4).[19]

In addition to the involvement of high-penetrance genes in UCC, the role of low-penetrance genetic susceptibility has to be considered. So far, research has focused on variants in genes involved in carcinogen metabolism. Only the *NAT* and especially the *GST* genes are consistently shown to modify the risk of UCC. More recent studies suggest a role of variants in other types of genes involved in base excision repair[20,21] and genes involved in cell adhesion such as E-cadherin.[22] Until recently, research in this genetic susceptibility has been performed in a small-scale gene-by-gene fashion. Future studies will have to evaluate combinations of genetic variants (and environmental factors) in a more high-throughput way using genome-wide (at least 200 000 SNPs will be necessary) or custom-made candidate gene chips.

Conflict of interest statement

None declared.

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Appendix 1

Likelihood-based segregation analysis was performed on the qualitative trait (affected or unaffected with UCC) using the REGTL program of the Statistical Analysis for Genetic Epidemiology (S.A.G.E.) software package.[10] The REGTL program allows for segregation analyses of a truncated trait with a logistic probability density function under a class A regressive model.[10,23] Class A regressive models assume that when a transmission of a phenotype is conditioned on the parents' genotypes, the offsprings' disease states are independent. Using the REGTL program two groups of models can be assumed. In the first group the presence (or absence) of a putative disease allele influences age at onset and in the second group it influences susceptibility. Because the risk of UCC increases with increasing age, age at onset was modelled. Individuals not diagnosed with UCC were censored at their age at time of filling out the questionnaire or age at death, if deceased. Assumptions of these analyses include Hardy-Weinberg equilibrium and autosomal inheritance of the phenotype through a single major locus with two alleles (bi-allelic locus).

UCC was defined as a dichotomous variable (Y), where Y = 1 if affected and Y = 0 if unaffected (censored). The frequency of the putative high-risk allele A was denoted as q_A. The transmission parameters (τ_i, where i represents an individual's type (AA, AB, BB)) represent the probability of a parent transmitting type A (in the case of genetic models, the A allele) to their offspring. Therefore, for genetic models, the transmission parameters τ_{AA}, τ_{AB} and τ_{BB} are fixed at 1 for individuals of type AA, 0.5 for individuals of type AB and 0 for individuals of type BB. In these models, age at onset is set to follow a logistic distribution with baseline parameter β and age coefficient α.[24] This symmetric distribution is similar to a normal distribution with mean $-\beta/\alpha$ and variance $\pi^2/3\alpha^2$. Susceptibility (γ) was also estimated, where susceptibility is defined as the cumulative probability of developing UCC if one lives to age infinity. The model can include covariates ($\mathbf{x}$) which coefficients are indicated by ζ_k for covariates k = 1 to N. In our model we included sex and smoking status (smoking status was defined as ever/never smoking). Then, the cumulative probability (or penetrance) that an individual

is affected by a certain age (age specific penetrance) was calculated for each type as:

$$P(Y \mid \text{genotype } i, \text{age}) = \gamma(e^{(\beta i+(\alpha*\text{age})+(\xi i*xi))})/(1+e^{(\beta i+(\alpha*\text{age})+(\xi i*xi))})$$

If the observed age at onset does not follow a logistic distribution, this model is still appropriate after transformation of age. The observed ages at onset were transformed according to:[25] $aG1 * \ln(\text{age})$, where aG1 is the geometric mean age at diagnosis of all relatives (age at time of questionnaire or age at death in case of non-affected relatives). Additionally, regressive familial effects that may indicate polygenic inheritance can be incorporated into the model. However, due to the limited number of affected individuals in our dataset (i.e. affected spouse pairs or affected mother-offspring or father-offspring pairs), such effects were not included in the analyses.

Because each family was identified through a single UCC case, ascertainment correction was performed by conditioning the likelihood of each pedigree on the proband's affection status using his age at onset as recorded.[26,27]

REFERENCES

1. Kiemeney LALM. Familial bladder cancer. In: Lerner SP, Schoenberg M, Sternberg C, editors. *Textbook of bladder cancer*. London: Taylor & Francis; 2006.
2. Kramer AA, Graham S, Burnett WS, Nasca P. Familial aggregation of bladder cancer stratified by smoking status. *Epidemiology* 1991;**2**:145–8.
3. Kiemeney LA, Moret NC, Witjes JA, Schoenberg MP, Tulinius H. Familial transitional cell carcinoma among the population of Iceland. *J Urol* 1997;**157**:1649–51.
4. Aben KKH, Witjes JA, Schoenberg MP, Hulsbergen-van de Kaa ALM, Verbeek ALM, Kiemeney LALM. Familial aggregation of urothelial cell carcinoma. *Int J Cancer* 2002;**98**:274–8.
5. Dong C, Hemminki K. Modification of cancer risks in offspring by sibling and parental cancers from 2,112,616 nuclear families. *Int J Cancer* 2001;**92**:144–50.
6. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med* 2000;**343**:78–85.
7. World Health Organization (WHO). *International Classification of Diseases for Oncology*. 1st ed. Geneva: WHO; 1976.
8. World Health Organization (WHO). *International Classification of Diseases for Oncology*. 2nd ed. Geneva: WHO; 1990.
9. Akaike H. A new look at the statistical model identification. *IEEE Trans Automatic Control* 1974;**AC-19**:716–23.
10. S.A.G.E. Statistical Analysis for Genetic Epidemiology, Release 3.0. *Computer package available from the Department of Epidemiology and Biostatistics*, Case Western Reserve University, Cleveland, OH, 1997.
11. Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH. Systemic population-based assessment of cancer risk in first-degree relatives of cancer probands. *J Natl Cancer Inst* 1994;**86**:1600–8.
12. Veltman JA, Schoenmakers EF, Eussen BH, Janssen I, Merkx B, van Cleef B, et al. High-throughput analysis of subtelomeric chromosome rearrangements by use of array-based comparative genomic hybridization. *Am J Hum Genet* 2002;**70**:1269–76.
13. Schoenberg M, Kiemeney L, Walsh PC, Griffin CA, Sidransky D. Germline translocation t(5;20)(p15;q11) and familial transitional cell carcinoma. *J Urol* 1996;**155**:1035–6.
14. Guo Z, Linn JF, Wu G, Anzick SL, Eisenberger CF, Halachmi S, et al. 1CDC91L1 (PIG-U) is a newly discovered oncogene in human bladder cancer. *Nat Med* 2004;**10**:374–81.
15. Schultz IJ, Kiemeney LA, Witjes JA, Schalken JA, Willems JL, Swinkels DW, et al. CDC91L1 (PIG-U) mRNA expression in urothelial cell carcinomas. *Int J Cancer* 2005;**20**(116):282–4.
16. Tarkkanen A, Karjalainen K. Excess of cancer deaths in close relatives of patients with bilateral retinoblastoma. *Ophthalmologica* 1984;**189**:143–6.
17. DerKinderen DJ, Koten JW, Nagelkerke NJ, Tan KE, Beemer FA, Den Otter W. Non-ocular cancer in patients with hereditary retinoblastoma and their relatives. *Int J Cancer* 1988;**41**:499–504.
18. Sanders BM, Jay M, Draper GJ, Roberts EM. Non-ocular cancer in relatives of retinoblastoma patients. *Br J Cancer* 1989;**60**:358–65.
19. Fletcher O, Easton D, Anderson K, Gilham C, Jay M, Peto J. Lifetime risks of common cancers among retinoblastoma survivors. *J Natl Cancer Inst* 2004;**96**:357–63.
20. Matullo G, Guarrera S, Carturan S, Peluso M, Malaveille C, Davico L, et al. DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. *Int J Cancer* 2001;**92**:562–7.
21. Stern MC, Umbach DM, van Gils CH, Lunn RM, Taylor JA. DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:125–31.
22. Zhang X, Ma X, Zhu QG, Li LC, Chen Z, Ye ZQ. Association between a C/A single nucleotide polymorphism of the E-cadherin gene promoter and transitional cell carcinoma of the bladder. *J Urol* 2003;**170**:1379–82.
23. Bonney GE. Regressive logistic models for familial diseases and other binary traits. *Biometrics* 1986;**42**:611–25.
24. Elston RC, George VT. Age at onset, age at examination, and other covariates in the analysis of family data. *Genet Epidemiol* 1989;**6**:217–20.
25. Box GEP, Cox DR. An analysis of transformations. *J R Stat Soc B* 1964;**26**:211–52.
26. Elston RC, Sobel E. Sampling considerations in the gathering and analyses of pedigree data. *Am J Hum Genet* 1979;**27**:31–45.
27. Cannings C, Thompson EA. Ascertainment in the sequential sampling of pedigrees. *Clin Genet* 1977;**12**:208–12.