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BRIEF COMMUNICATION

Increased risk of skin cancer in Japanese heterozygotes of xeroderma pigmentosum group A

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Abstract

This study was designed to learn if asymptomatic heterozygotes with mutations in a DNA repair gene are at an increased risk for cancer. To examine this, we focused on carriers of an *XPA* founder mutation because the frequency of xeroderma pigmentosum (XP) patients is much greater among Japanese than Caucasians, more than half of Japanese XP patients are affected at the *XPA* gene, and the majority of XP-A patients carry the same founder mutation in the *XPA* gene. Here we show that the frequency of *XPA* heterozygote was 14/1698 (0.8%) in cancer-free controls, and the corresponding frequency in patients with nonmelanocytic skin cancer that developed in sun-exposed areas was 11/440 (2.5%, OR = 3.08, $p = 0.0097$) for basal cell carcinoma, and 3/272 (1.1%, OR = 1.34, $p = 0.72$) for squamous cell carcinoma. These results suggest a moderately elevated risk for skin cancer among *XPA* heterozygotes.

Introduction

Xeroderma pigmentosum (XP) is an autosomal recessive disorder wherein affected individuals have an extremely high risk (1,000–10,000 times) of developing skin cancer in sun-exposed areas [1]. This high risk of developing skin cancer is attributed to a defect in repairing ultraviolet light-induced DNA damage, and there are eight DNA repair genes involved in XP; i.e., from *XPA* to *XPG*, and *POLH* (an XP variant) [1, 2]. Although the frequency of homozygous XP patients is quite low, on the order of 10^{-5}, the frequency of heterozygotes should be much higher; e.g., on the order of 10^{-2}. In other words, there is a large number of heterozygotes in the population, and hence even if their excess relative risk for cancer might be small, their burden on the health-care system might not be negligible. In the past, cancer risks for *XP* heterozygotes were suggested to be elevated [3, 4].

To evaluate cancer risks for heterozygotes, the Japanese population offers unique advantages: the frequency of XP patients is an order of magnitude higher than in Caucasians [5], more than half of Japanese XP patients are affected at the *XPA* gene locus (XP-A patients), about 80% of XP-A patients are homozygous for a specific founder mutation, and 95% of XP-A patients bear at least one allele with the founder mutation [6]. This founder mutation causes frame

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shift and no protein production, and can be easily detected using the PCR-restriction fragment length polymorphism (PCR-RFLP) [7]. Our previous study revealed that about 1% of the Japanese population residing in Hiroshima and Nagasaki areas are heterozygous carriers with this founder mutation [8]. Here we report the frequency of the mutation carrier among skin cancer patients.

Materials and methods

We obtained 928 paraffin-embedded blocks of non-melanocytic skin cancers (NMSCs) from three hospitals around Hiroshima city (collection period: 1957–2011). Among these, 545 were basal cell carcinoma (BCC) and 383 were squamous cell carcinoma (SCC). None of them are cohort members of epidemiologic studies of atomic bomb survivors. One whole section (5 µm thick) was used for DNA extraction and screening purposes. When a suspected carrier of the *XPA* founder mutation was found, DNA extraction processes were conducted anew to obtain DNA samples from tumor and non-tumor parts separately by using the laser-microdissection system (AS-LMD; Leica Microsystems Japan, Tokyo). For control samples, 678 lymphocyte slides from offspring of atomic bomb survivors with minimum dose exposures (<10 mGy) in Hiroshima were newly obtained and used as described previously [8]. PCR-RFLP methods for screening the founder mutation allele were described previously (Fig. 1) [8]. Fisher's exact tests were used to determine whether the frequency of the founder mutation heterozygote differed between the control population and the BCC and SCC cases, as well as in subtypes. The present study was approved by the Ethics Committees at each hospital and at the Radiation Effects Research Foundation.

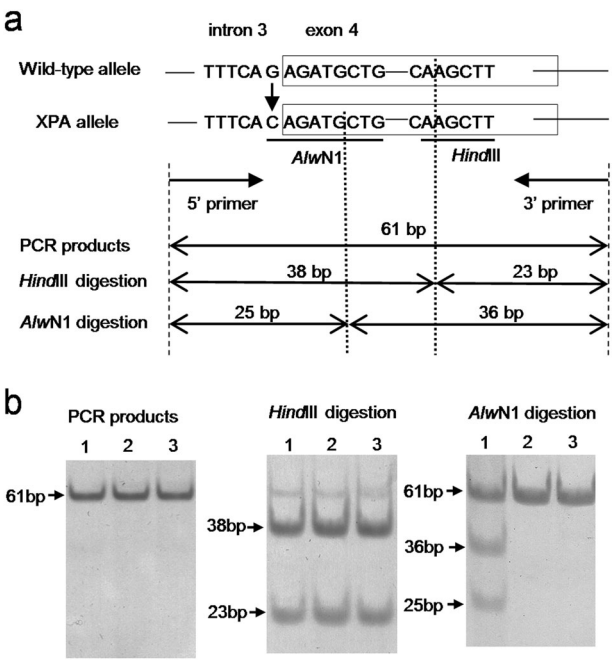

Fig. 1 **a** A schema to distinguish the mutant *XPA* allele from wild-type allele: PCR products (61 bp) were digested with *Hind* III enzyme, which gave rise to 38-bp and 23-bp fragments for either allele. On the other hand, digestion with *Alw*N1 produces 25-bp and 36-bp fragments only when the PCR products contained the mutant *XPA* allele that has C instead of G at the splicing junction. Thus, in case of *XPA* heterozygotes bearing the mutant allele, only half of the PCR products are digested with the *Alw*N1 enzyme, giving rise to three fragments of different sizes; i.e., 25-bp, 36-bp, and 61-bp. **b** Gel electrophoresis of PCR-RFLP products with *Alw*N1 or *Hind* III digestion. *XPA* heterozygote, lane 1; normal individual (both alleles are wild-type), lanes 2, 3

Results and discussion

We have previously identified 9 carriers with the *XPA* founder mutation among 1020 control individuals (the mean age at blood collection was 24 years old) [8]. In the present study, we screened additional 678 individuals and found 5 new carriers, giving a total frequency of 14 carriers among 1698 individuals (0.82%) in the pooled Hiroshima and Nagasaki populations. Compared to the control frequency, we found 16 suspected carriers (Table 1) among 928 BCC or SCC cases in the first screening. Subsequent tests for DNA samples from tumor and non-tumor parts showed that all 16 cases had the founder mutation in both tumor and

Table 1 Type of skin cancer, location, gender, and age of the patient at the time of operation for the 16 heterozygotes with the *XPA* founder mutation detected among 928 skin cancer patients with BCC or SCC

Patient	Type	Location	Sun-exposed	Sex	Age at operation
SC1	BCC	Lip	Yes	F	64
SC2	BCC	External ear	Yes	M	74
SC3	BCC	Face	Yes	M	75
SC4	BCC	Face	Yes	M	79
SC5	BCC	Face	Yes	M	81
SC6	BCC	Face	Yes	M	Unknown
SC7	BCC	Face	Yes	F	47
SC8	BCC	Face	Yes	F	75
SC9	BCC	Face	Yes	F	82
SC10	BCC	Face	Yes	F	87
SC11	BCC	Scalp and neck	Yes	M	61
SC12	BCC	Trunk	No	F	83
SC13	SCC	External ear	Yes	M	73
SC14	SCC	Face	Yes	F	87
SC15	SCC	Scalp and neck	Yes	M	82
SC16	SCC	Trunk	No	M	59

BCC basal cell carcinoma, *SCC* squamous cell carcinoma, *M* male, *F* female

Table 2 Number of heterozygotes with the *XPA* founder mutation in each type of skin cancer

Type of skin cancer	No. of samples	No. of carriers	*p* value	OR (95% CI)
Total no. of BCC and SCC	928	16 (1.7%)	0.053	2.11 (0.96–4.9)
BCC	545	12 (2.2%)	0.018	2.71 (1.14–6.35)
SCC	383	4 (1.0%)	0.758	1.27 (0.303–4.07)
Total no. in sun-exposed areas	712	14 (2.0%)	0.022	2.41 (1.06–5.49)
BCC	440	11 (2.5%)	0.0097	3.08 (1.26–7.37)
SCC	272	3 (1.1%)	0.72	1.34 (0.246–4.85)
Control group	1698	14 (0.82%)		Referent

OR odds ratio, *CI* confidence interval

non-tumor parts. This resulted in a carrier frequency of 1.7% (Table 2), which was borderline significant (the odds ratio [OR] was 2.11; 95% confidence interval [CI] 0.96–4.9, $p = 0.053$, Table 2). For cancers which developed in sun-exposed areas (Supplementary Table S1), 14 out of 712 were carriers (2.0%) with an OR of 2.41, which was significant (CI 1.06–5.49, $p = 0.022$, Table 2). By histological types, the OR was significantly higher only for BCC (11/440 or 2.5%; OR = 3.08, CI 1.26–7.37, $p = 0.0097$). The mean age at operation was 68.2 years old (±SD 14.1) for all BCCs and 74.3 years old (±SD 12.8) for all SCCs while the corresponding ages of *XPA* heterozygotes were 73.5 and 75.3 years old for BCC and SCC, respectively, which provided no indication for earlier onset of cancer among the heterozygotes. Overall, present results indicate that carriers of the *XPA* founder mutation are at higher risk of developing BCC in sun-exposed parts of the skin than non-carriers. For SCC, the elevated OR of 1.34 was smaller than for BCC (Table 2) and not significant possibly due to the smaller number of cases.

There are several limitations with the present data. The present study was not of traditional case-control design and there are differences between the populations from which the two groups were sampled. The mean age of the control group was the early 20 s while it was over 60 in the skin cancer group (Table 1); however, the age difference is unlikely to have causally affected the results as it is difficult to imagine that the prevalence of mutation carriers in a population increases with increasing age due to their better survival. We must also assume that there has not been a substantial shift in the prevalence of heterozygotes in this population, which is reasonable over such a short time period. Although the cancer cases and controls were not matched for residential areas, background rates for BCC and SCC were similar in Hiroshima and Nagasaki [9] with the frequency of *XPA* heterozygote being also similar in the two areas, i.e., 9/1190 in Hiroshima vs. 5/508 in Nagasaki ($\chi^2 = 0.23$, $p = 0.63$), respectively.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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