OBSERVATION

Multiple Primary Melanomas in a *CDKN2A* Mutation Carrier Exposed to Ionizing Radiation

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Background: Recent research has shown a possible causal relationship between ionizing radiation exposure and melanoma. Individuals with mutations in *CDKN2A* (cyclin-dependent kinase inhibitor 2A), the major melanoma predisposition gene, have an increased susceptibility to melanoma-promoting exposures, such as UV light. We describe a patient from a familial melanoma pedigree with 7 primary melanomas on the right side of her body, the first occurring 5 years after exposure to atmospheric nuclear bomb testing in the 1950s.

Observations: Physical examination revealed phototype I skin, red hair, and 26 nevi (14 on the right and 12 on the left side of her body). One nevus was larger than 5 mm, and 2 were clinically atypical. Sequence analysis demonstrated a known deleterious mutation in *CDKN2A* (G–34T) and homozygosity for a red hair color variant in *MC1R* (melanocortin 1 receptor) (R151C). Fluorescence in situ hybridization analysis of blood, fibroblasts, and melanocytes from both upper extremities ruled out mosaicism.

Conclusions: Individuals such as this patient, who has *CDKN2A* and *MC1R* mutations, are likely to be more susceptible to environmental insults. A careful review of environmental exposures in these vulnerable cases may reveal cancer-promoting agents, such as ionizing radiation, that go unnoticed in less susceptible populations.

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RISK FACTORS FOR MELANOMA include environmental insults (eg, blistering sunburns) and genetic factors underlying hair and eye color, skin type, many or atypical moles, and family history. Being a member of a melanoma-prone family is the most significant risk factor, increasing one's likelihood of developing a melanoma to 35 to 70 times that of the general population.[1] The gene for cyclin-dependent kinase inhibitor 2A (*CDKN2A* or *p16*) is the major melanoma predisposition gene identified at present. p16 is a key regulator of G_1/S cell cycle entry by controlling phosphorylation of retinoblastoma via inhibition of cyclin-dependent kinases 4 and 6 (CDK4 and CDK6).[2] Variants in the gene for the melanocortin 1 receptor (*MC1R*) alter the production of pigment, shifting the equilibrium from eumelanin to pheomelanin. *MC1R* is a low-penetrance melanoma susceptibility gene; heterozygotes for the *R151C* allele have a 2-fold increased risk, and homozygotes such as the patient described herein carry an approximate 4-fold risk to develop melanoma compared with the general population.[3] Carriage of *MC1R* variants by those with the *CDKN2A* mutation confers an additional risk for melanoma development.[4-7]

The relationship between environmental and genetic factors is illustrated by international investigations of *CDKN2A* mutation carriers. The GenoMel, an International Melanoma Genetics Consortium (http://www.genomel.org/), reported that the lifetime prevalence of melanoma among individuals with *CDKN2A* mutations varied considerably by geographic locale.[8] The lifetime incidence of melanoma was 58% in Europe, 76% in the United States, and 91% in Australia among *CDKN2A* carriers of similar ethnic backgrounds. Bishop et al[8] suggested that this difference was best explained by the difference in UV exposure and UV sensitivity among the different populations. The *CDKN2A* mutation carrier population clearly represents the group most likely to develop melanoma, and through careful history and observation, researchers may be able to deduce other melanoma-promoting events that may not be obvious in the general population.

Ionizing radiation is a known mutagen and carcinogen. Long- and short-term exposures have been associated with leukemia and thyroid, breast, and lung

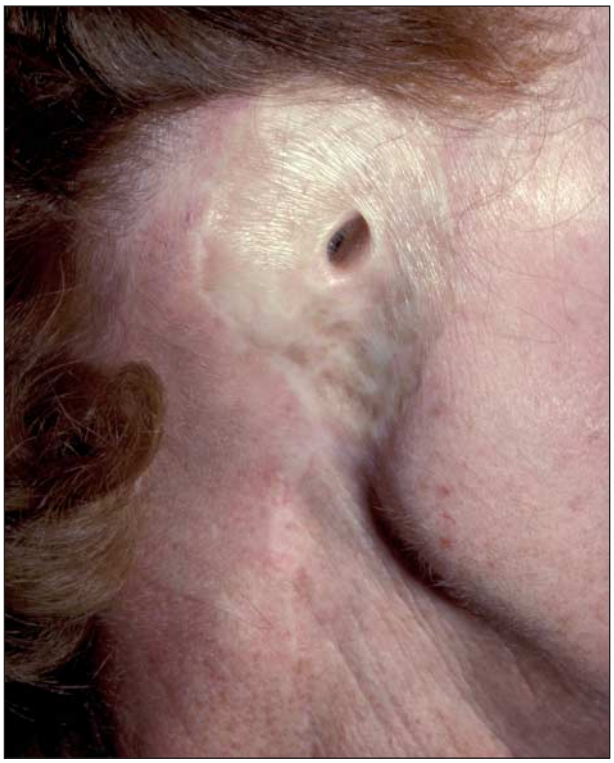


Figure 1. Right side of the patient's face at the time she underwent evaluation in our clinic. The right external ear had been removed approximately 40 years previously owing to melanoma and it healed well. The patient's phototype I skin, red hair, and external auditory canal are apparent.

cancers.[9,10] Studies that tracked the rates of cancer in the atomic bomb survivors of Nagasaki and Hiroshima reported an increased rate of basal cell and squamous cell carcinomas, but not melanoma, among this exposed Japanese population. In 2005, Fink and Bates[11] published a meta-analysis of melanoma in 7 cohorts of individuals with short- or long-term exposure to ionizing radiation. Most of these groups included large white populations, the race most susceptible to melanoma development, and not represented by the work on Nagasaki and Hiroshima survivors. The authors reported a statistically significantly increased relative risk of melanoma in 5 of their 7 cohorts, including a cohort of 3702 subjects involved in the 1957 above-ground nuclear testing.[11]

A large, retrospective analysis of individuals treated with ionizing radiation for hereditary retinoblastoma demonstrated a significantly elevated rate of melanomas in the treatment field.[12] This would suggest that in genetically predisposed individuals, exposure to ionizing radiation can promote melanoma through the retinoblastoma pathway. The *CDKN2A* protein product, p16, regulates cell growth through the retinoblastoma pathway,[13] but the effects of ionizing radiation on *CDKN2A* mutation carriers have yet to be studied. In a 2005 study of multiple primary melanomas (MPM) and *CDKN2A* mutations, Puig et al[14] noted that one of their patients with MPM had a *CDKN2A* mutation and a history of long-term exposure to ionizing radiation. We herein describe a *CDKN2A* mutation carrier with 7 primary melanomas on the same side of her body and a unique history of exposure to ionizing radiation.

OBSERVATIONS

The patient is a white woman who underwent investigation for a history of MPM. Medical records and blood and tissue samples were collected for investigation.

Her medical history was remarkable for the development of a melanoma on her right ear at 28 years of age, treated by excision and a radical neck lymph node dissection (**Figure 1**). She subsequently developed 6 additional primary melanomas. Of the 7 total melanomas, 6 were found on the right side of her body and 1 was in the midline (**Figure 2**). A squamous cell carcinoma of her right upper lip was diagnosed in 1997. Other pertinent medical history includes a 46-year history of smoking, coronary artery disease with placement of 3 coronary artery stents, and polycythemia vera diagnosed at 63 years of age.

Her environmental exposure history was significant for 1 blistering sunburn before 20 years of age, with minimal sunbathing and no subsequent sunburns. She also reports 1 episode of intense exposure to ionizing radiation at 27 years of age that occurred in October 1958 in southern Utah and Nevada. The patient and her husband left the car in which they were traveling to observe an atmospheric nuclear detonation from an undetermined distance for 20 to 30 minutes. After leaving the blast site, the patient sat in the passenger side of the car, the side oriented toward the test site, riding with her arm outside the car. They noted a fine, sticky dust on the top of the car when they arrived home, consistent with nuclear fallout. The patient denies the development of nausea or other acute symptoms the day of the exposure.

Her family history includes a cousin with melanoma and melanoma in the children of another cousin. Multiple other cancer types were also described in her extended family.

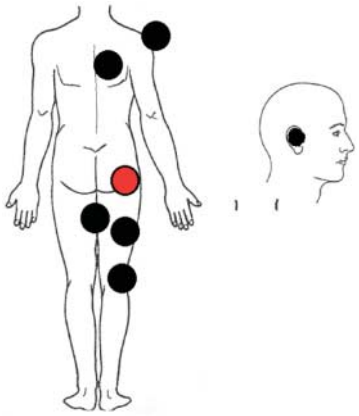
PHYSICAL EXAMINATION

Pertinent findings included phototype I skin, green eyes, red hair, and mild freckling. On the right side of her body, the patient had 14 moles, 1 of which was atypical. No nevi were larger than 5 mm. She also had 12 well-healed biopsy and treatment scars. On the left side of her body, the patient had 12 moles, 1 larger than 5 mm and 1 with atypical features, and 5 well-healed biopsy scars.

LABORATORY TESTS

Results of routine laboratory tests, including complete blood cell count, liver function tests, and a lipid panel, were unremarkable other than an elevated hematocrit consistent with polycythemia vera. Sequencing of the *CKDN2A* gene and promoter region revealed a G–34T 5′ UTR mutation known to be deleterious.[15] No additional mutations were found within the coding region. The alternate reading frame gene was also sequenced and had no mutations. Sequencing of *MC1R* demonstrated that the patient was homozygous for the *R151C* mutation. Chromosomal studies were performed on melanocytes, peripheral blood, and fibroblasts. Tissue specimens were collected from both sides of the body. Metaphase cells analyzed from cultures of all these tissues revealed a nor-

A



B

Laterality	Location	Diagnosis
Right	Ear	MM
Right	Leg	MM
Right	Thigh	SSM in situ
Left	Inner thigh	SSM in situ
Not applicable	Middle upper back	SSM
Right	Popliteal fossa	SSM
Right	Upper lateral arm	MM in situ

Figure 2. The location of the melanoma sites in the patient. A, Locations are approximated by the dots. The red dot indicates a melanoma that occurred on the anterior surface of the participant. B, Location and diagnosis of melanoma. MM indicates melanoma; SSM, superficial spreading melanoma.

mal female karyotype. Fluorescence in situ hybridization of all specimen types was performed, and no chromosomal abnormalities were found. Direct sequencing of *MC1R* was performed as previously described.[4,16,17]

COMMENT

This is a unique case report of ionizing radiation exposure in an individual with a deleterious *CDKN2A* mutation and MPM. The patient had 7 primary melanomas during a course of about 30 years, starting late in her third decade of life. Her laboratory studies are significant for a deleterious *CDKN2A* mutation, homozygosity for an *MC1R* red hair color variant, and fluorescence in situ hybridization results that were negative for chromosomal aberrancy on either side of her body. Her physical examination revealed skin phototype I, red hair, numerous biopsy and excisional scars, and a random distribution of 26 nevi, 2 of which had atypical features. Most of her melanomas occurred in traditionally sun-protected areas. Her family history is consistent with hereditary melanoma.[1]

Many groups have investigated the correlation between MPM and *CDKN2A* mutations (**Table**).[14,18-24] Collectively, these groups have described 467 individuals with MPM, 70 of them (15.0%) with *CDKN2A* mutations. Not all groups specified the number of primary melanomas for each individual. Of the studies that did specify the number of primary melanomas,[14,18,19,23,24] 5 of 311 cases (1.6%) had at least 7 primary melanomas. Even among individuals with *CDKN2A* mutations, it is rare to have 7 primary melanomas. Puig et al[14] reported that among their 104 patients with MPM, only 3 had a second primary melanoma appear in proximity (defined as on the same extremity as the first melanoma).[14] Our patient was initially seen with a grouping of 4 independent melanomas on the right side, 1 on the inner left thigh, and 3 melanomas close to one another on the right ear, right shoulder, and upper back area (Figure 2).

Her exposure history is significant for 1 blistering sunburn and her report of observing an above-ground nuclear test while traveling in Utah and Nevada. The time of the exposure she describes is chronologically consistent with Operation Hardtack II (September 12 to October 30, 1958), during which atmospheric nuclear testing took place in southern Nevada.[25,26]

Previous studies have linked exposure to ionizing radiation and nonmelanoma skin carcinomas, including leukemia and thyroid, breast, and lung cancers.[9,27,28] There are also several brief reports that have described an excess rate of polycythemia rubra vera in subjects exposed to the fallout of nuclear tests.[29,30] Ionizing radiation has not been historically considered a promoting agent for melanoma, because many of the original studies that reviewed the cancer in atomic blast survivors in Japan did not identify melanoma as an outcome of interest.

Table. Overview of Studies Published on *CDKN2A* Testing in Patients With MPMs

Source	Total MPM Cases, No.	Characteristics of MPM Cases (Cases With *CDKN2A* Mutations, No.)
Puig et al,[14] 2004	104	1 Case, 7 PM (1); 2 cases, 6 PM (1); 1 case, 5 PM (1); 5 cases, 4 PM (2); all other cases, ≤3 PM (12)
Blackwood et al,[18] 2002	96	1 Case, 9 PM (0); 1 case, 7 PM (1); 2 cases, 6 PM (1); 2 cases, 5 PM (0); 4 cases, 4 PM (1); all other cases, ≤3 PM (6)
Hashemi et al,[19] 2000	80	1 Case, 6 PM (0); 2 cases, 5 PM (0); 4 cases, 4 PM (2); all other cases, ≤3 PM (7)
Monzon et al,[20] 1998	33	3 Cases, >3 PM (1); all other cases, ≤3 PM (4)
Auroy et al,[21] 2001	100	4 Cases, ≥5 PM (3); 2 cases, 4 PM (0); all other cases, ≤3 PM (6)
Mantelli et al,[22] 2002	23	3 Cases, ≥3 PM (3); all other cases, 2 PM (8)
Mackie et al,[23] 1998	17	1 Case, 8 PM (1); No. of PM in other cases not stated (1)
Eliason et al,[24] 2006	14	1 Case, 7 PM (1); all other cases, ≤3 PM (7)
Total	**467**	70 Cases, MPM (70)

Abbreviations: MPM, multiple primary melanomas; PM, primary melanomas.

The studies reviewed by Fink and Bates[11] collectively totaled hundreds of thousands of individuals who were predominantly white, the population most susceptible to melanoma. In addition to the rates of melanoma, the authors reported which of these studies demonstrated an excess rate of leukemia as a means to distinguish which cohorts received a significant radiation exposure. Five of the 7 cohorts had elevated rates of leukemia and melanoma. The remaining 2 cohorts did not have excess melanoma or excess leukemia, suggesting that both did not receive sufficient irradiation to induce any malignancy. They conclude that melanoma has not always been considered a significant end point in epidemiological studies on ionizing radiation, which may have hampered identifying a potentially causal relationship between ionizing radiation and melanoma. They also recommend that future epidemiological studies of ionizing radiation consider inclusion of melanoma as an outcome of interest.[11]

It is not clearly understood why some patients with *CDKN2A* mutations never develop melanoma, whereas others with the same mutation and even in the same family seem exquisitely prone to the development of numerous primary lesions. We suggest that in our patient, the MPM may have been a result of the superimposition of environmental exposures (ionizing radiation) on her highly vulnerable genetic predisposition. The careful examination of phenotypic and environmental risk factors in the very vulnerable subpopulation of *CDKN2A* mutation carriers may be a strategy to identify and clinically verify risk factors less obvious in the general population.

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Author Contributions: Dr Leachman had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Hansen, Bowen, Cannon-Albright, and Leachman. *Acquisition of data*: Eliason, Hart, Florell, Harris, Swinyer, and Leachman. *Analysis and interpretation of data*: Eliason, Porter-Gill, Chen, Sturm, and Leachman. *Drafting of the manuscript*: Eliason, Hansen, Hart, and Leachman. *Critical revision of the manuscript for important intellectual content*: Eliason, Hansen, Hart, Porter-Gill, Chen, Sturm, Bowen, Florell, Harris, Cannon-Albright, Swinyer, and Leachman. *Administrative, technical or material support:* Chen and Sturm. *Study supervision*: Leachman.
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