Nevus Distribution in a Utah Melanoma Kindred with a Temperature-Sensitive *CDKN2A* Mutation

To the Editor:

We recently reported in the *Journal* our longitudinal phenotypic observations of a well-characterized Utah melanoma kindred carrying a temperature-sensitive mutation (V126D) in *CDKN2A* (Florell *et al*, 2004). The V126D mutation, which co-segregates with melanoma susceptibility (Kamb *et al*, 1994), results in a p16 protein with diminished capacity to bind CDK4/CDK6, inhibit Rb phosphorylation, and induce exit from the cell cycle (G1 arrest) at physiologic temperatures (Parry and Peters, 1996). As this mutation was associated with increased nevus size and number in this family (Meyer *et al*, 1992; Florell *et al*, 2004), we hypothesized that given its temperature sensitivity it might also be associated with excess formation of nevi and/or melanomas on warmer regions of the body, defined as the head, neck, and trunk. Here we report nevus and melanoma distribution data derived from 29 family members and 11 spouse control subjects over an average interval of 15 y.

This study was approved by the Institutional Review Board at the University of Utah and was conducted according to Declaration of Helsinki principles. All subjects agreed to participate and informed written consent was obtained. The subjects were not aware of their *CDKN2A* mutation status. A total of 13 V126D mutation carriers and 16 non-carriers were examined, with 11 married-in spouses serving as a control group. All participants were examined in this study and initially 15 y ago by the same dermatologist (L. J. M.), who was also unaware of subject mutational status. The initial and follow-up examinations were conducted in a similar fashion and included a total body skin exam in which location and size of all nevi ≥ 2 mm in diameter were recorded on a body map diagram as described (Florell *et al,* 2004). The head, neck, and trunk were considered "warm" regions and the extremities "cold" regions. Invasive melanomas were confirmed by the Utah Cancer Registry, by obtaining the pathology report, and/or by review of histologic slides by dermatopathologists (S. R. F. and R. M. H.). The nevus count and nevus density data were analyzed using multiple linear regression with appropriate differences, constructed for each subject, as response variables. Carrier status, age at first visit, and gender were used as explanatory variables in the multiple regression analysis.

Nevus distribution data were analyzed by absolute number of nevi and by the mean change of nevi in warm and cold regions over time. As shown in Fig 1, mutation carriers developed more nevi on warm regions than non-carrier or spouse control subjects ($p = 0.004$), but the carriers, non-carriers, and spouse control subjects showed a similar rate of nevus development on cold regions ($p = 0.07$). Because *CDKN2A* mutation carriers are more prone to nevus development in this family (Meyer *et al,* 1992; Florell *et al,* 2004), we also evaluated nevus distribution based on the mean change of nevi and nevus density in warm and cold regions over the 15 y follow-up period. In this way, we minimized the possibility that the preponderance of warm-region nevi in *CDKN2A* mutation carriers was merely reflective of a nevogenic effect of *CDKN2A* mutation. Mutation carriers demonstrated a significant increase in the mean change of nevus number and density on the warm regions, as compared with the non-carriers and spouse control subjects. The mean change in these parameters was not significantly different in the cold regions (Table I). Four V126D *CDKN2A* mutation carriers developed 11 invasive melanomas, six on warm regions (55%) and five on cold regions (45%), which was not statistically significant. A significant difference in nevus distribution was not observed in a second Utah melanoma kindred with a promoter-region *CDKN2A* mutation (−34 G>T) over a 15-interval (12 mutation carriers, 11 non-carriers, and 11 spouse control subjects; Fig 1).

There is precedent for temperature-sensitive mutations resulting in a demonstrable clinical phenotype. The well-known temperature-sensitive tyrosinase mutation ("Siamese cat mutation") occurs in both cats and mice, producing an increased relative pigmentation in the cooler areas of the body such as the tips of the ears, tail, and paws (Searle, 1968). An analogous human tyrosinase mutation confers a temperature-dependent distribution of pigmentation in human carriers (Giebel *et al,* 1991; King *et al,* 1991). In addition, although skin temperature regulation is complex and depends on thermoregulatory and environmental factors (Houdas and Ring, 1982; Wenger, 1995), the normal fluctuations in body temperature appear to encompass a range that would impact V126D *CDKN2A* function. Studies measuring skin temperature in warm, neutral, and cold conditions suggest that core regions (head and trunk) are, on average, warmer than the extremities in neutral (26°C–27°C) and cold environments (Folk, 1974; Webb, 1992; Wenger, 1995), but skin temperature in warm environments (34°C) is similar over the entire integument (Folk, 1974). Temperature measurements of skin in a comfortable environment are slightly higher in warm body regions and have been measured at 34°C–35°C as compared with 31°C–34°C in the cooler extremities (Houdas and Ring, 1982; Webb, 1992). Thus, the

Abbreviations: TND, total nevus density; TNN, total nevus number

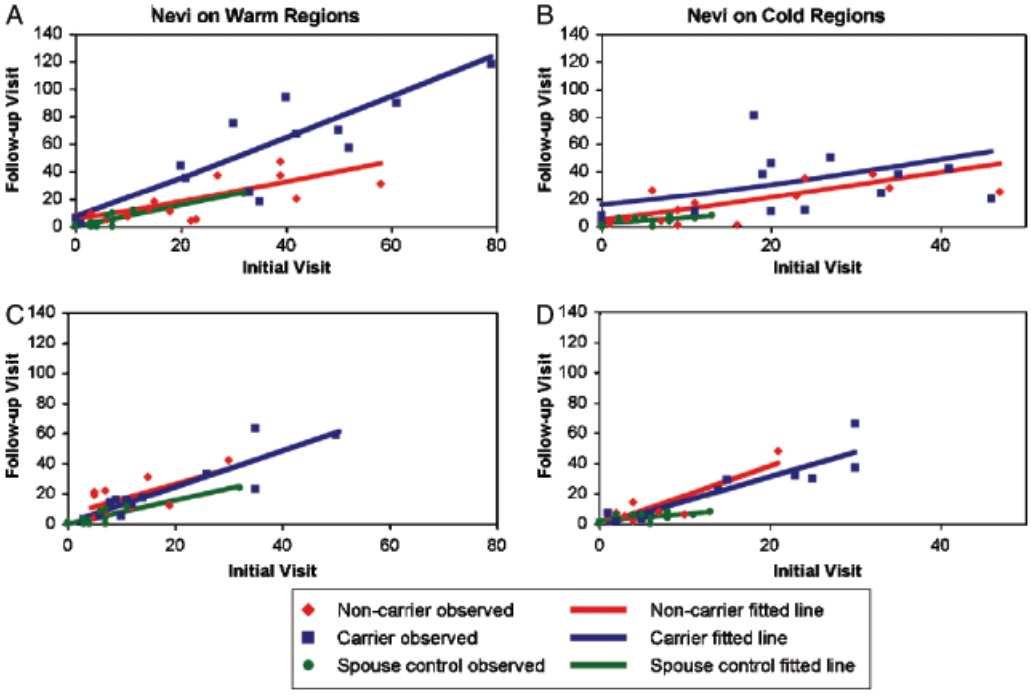

Figure 1
V126D mutation carriers develop more nevi on warm regions of the body. These plots of nevus number at the initial (x coordinate) and follow-up (y coordinate) examinations demonstrate an increased number of nevi on warm areas in V126D CDKN2A mutation carriers with a more pronounced increase in nevi at the follow-up visit. The rate of increase in the number of nevi is significantly greater in the carriers than non-carriers or spouse control subjects, after adjusting for the initial number of nevi ($p = 0.004$). A significant difference in the rate of nevus development on cold regions was not identified among the groups ($p = 0.07$) ((*A*, *B*), V126D CDKN2A kindred). A second Utah melanoma kindred with a promoter region mutation (−34 G > T) fails to demonstrate a significant difference in nevus distribution in the warm or cold regions (*C*, *D*) over a 15-y interval ($p = 0.62$ for warm regions; $p = 0.55$ for cold regions).

temperature range at which the V126D *CDKN2A* mutation is functionally impaired coincides with the range of normal fluctuations of cutaneous temperature.

Our data demonstrate a statistically significant disparity in the nevus distribution between mutation carriers and non-carriers, suggesting that the V126D temperature-sensitive mutation may influence the distribution of nevi in these subjects. We considered other explanations for the discrepancy observed in mutation carriers. Distribution of nevi is known to correlate with UV exposure, sunburn history, and phenotypic constitutional features (Kopf *et al,* 1985, 1986; Slade *et al,* 1995; Harrison *et al,* 1999; Autier *et al,* 2001; Carli *et al,* 2002). Significant differences between the genotypic groups, however, were not identified with respect to markers of chronic UV radiation (rhytides, poikiloderma, actinic keratoses, solar lentigines, etc.), self-reported sunburn history, or phenotypic constitutional features (hair and eye color, skin type) (Florell *et al,* 2004). We also considered the possibility that nevogenic *CDKN2A* mutations might induce nevus formation on the head, neck, and trunk preferentially, unrelated to temperature sensitivity. Nevus distribution data from a second Utah melanoma kindred with a promoter region *CDKN2A* mutation (−34 G > T) over a 15-y interval, however, showed no significant differences in nevus distribution between the genotypic groups.

In summary, we found that a temperature-sensitive *CDKN2A* mutation is associated with an altered nevus phenotype with increased rate of nevus development and

Table I. Mean change in nevus number and density in V126D CDKN2A mutation kindred

Region[a]	Group[b]	TNN[c] mean change initial *versus* F/U	95% CI	p[d]	TND[e] mean change initial *versus* F/U	95% CI	p[d]
Warm	Carrier	10	2–25	0.002	61	15–138	0.005
	Non-carrier	−1	−6 to 0		0	−15 to 7	
	Spouse	0	−4 to 4		0	−14 to 24	
Cold	Carrier	2	0–11	0.36	15	1–50	0.26
	Non-carrier	0	−2 to 3		1	−3 to 12	
	Spouse	0	−4 to 4		1	7–18	

[a]Warm regions of the body were defined as the head, neck, and trunk, and cold regions were defined as the upper and lower extremities.
[b]Subject mutation status was verified by sequencing the promoter region and exons 1α–3 of *CDKN2A* and exon 1β (*ARF*). A total of 13 V126D mutation carriers and 16 non-carriers were examined, with 11 spouses serving as a control group.
[c]Change in TNN represents difference in number of clinically detectable nevi $\geq$ 2 mm in diameter (# nevi at follow-up–# nevi at initial visit).
[d]The analysis used a square-root transformation to normalize the data, which were adjusted for age and sex. Statistical analysis was performed using Statistica 6.0 (StatSoft Inc., Tulsa, Oklahoma).
[e]Change in TND calculated by dividing area of all nevi by estimated body surface area (nevus density at follow-up–nevus density at first visit) (Goldgar *et al*, 1991; Meyer *et al*, 1992).
TNN, total nevus number; TND, total nevus density.

density on warm body regions among mutation carriers. Although there were slightly more melanomas on the warm areas, the difference was not significant. These findings provide the first *in vivo* evidence that a temperature-sensitive *CDKN2A* mutation may confer a temperature-dependent nevus distribution.

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This work was supported by grants from The Skin Cancer Foundation (S. R. F.), the Dermatology Foundation Leaders Society Dermatologist Investigator Research Fellowship and Clinical Career Development Award (S. R. F.), National Institutes of Health grants K23 RR17525-01 (S. R. F.), R01 CA102422 (L. A. C.), K08 AR48618 (D. G.) and R01 AR50102 (D. G.), Doris Duke Charitable Foundation (S. A. L.), Fellowship-To-Faculty Transition Award from the University of Utah, funded in part by the Howard Hughes Medical Institute (S. A. L.), The Huntsman Cancer Foundation (S. A. L., D. G.), the Tom C. Mathews Jr. Familial Melanoma Research Clinic at Huntsman Cancer Institute, Huntsman General Clinical Research Center Public Health Service grant (MO1 RR00064), National Cancer Institute (NCI) Cancer Center Support grant 5P30CA420-14, and the Utah Cancer Registry, funded by Contract # NCI-CN-67000 from the NCI with additional support from the Utah Department of Health and the University of Utah. We wish to thank Huntsman Cancer Foundation for database support provided to the Pedigree and Population Resource of Huntsman Cancer Institute. We gratefully acknowledge the willing participation of all the family members in this study.

DOI: 10.1111/j.0022-202X.2005.23945.x

Manuscript received May 24, 2005; revised July 6, 2005; accepted for publication July 7, 2005

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References

Autier P, Boniol M, Severi G, *et al*: The body site distribution of melanocytic naevi in 6–7 year old European children. Melanoma Res 11:123–131, 2001

Carli P, Naldi L, Lovati S, La Vecchia C: The density of melanocytic nevi correlates with constitutional variables and history of sunburns: A prevalence study among Italian schoolchildren. Int J Cancer 101:375–379, 2002

Florell SR, Meyer LJ, Boucher KM, *et al*: Longitudinal assessment of the nevus phenotype in a melanoma kindred. J Invest Dermatol 123:576–582, 2004

Folk GE: Principles of temperature regulation. *Textbook of Environmental Physiology*. Philadelphia: Lea & Febiger, 1974; p 88–132

Giebel LB, Tripathi RK, King RA, Spritz RA: A tyrosinase gene missense mutation in temperature-sensitive type I oculocutaneous albinism. A human homologue to the Siamese cat and the Himalayan mouse. J Clin Invest 87:1119–1122, 1991

Goldgar DE, Cannon-Albright LA, Meyer LJ, Piepkorn MW, Zone JJ, Skolnick MH: Inheritance of nevus number and size in melanoma and dysplastic nevus syndrome kindreds. J Natl Cancer Inst 83:1726–1733, 1991

Harrison SL, Buettner PG, MacLennan R: Body-site distribution of melanocytic nevi in young Australian children. Arch Dermatol 135:47–52, 1999

Houdas Y, Ring EFJ: Temperature distribution. *Human Body Temperature. Its Measurement and Regulation*. London: Plenum Press, 1982; p 81–103

Kamb A, Shattuck-Eidens D, Eeles R, *et al*: Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. Nat Genet 8:23–26, 1994

King RA, Townsend D, Oetting W, Summers CG, Olds DP, White JG, Spritz RA: Temperature-sensitive tyrosinase associated with peripheral pigmentation in oculocutaneous albinism. J Clin Invest 87:1046–1053, 1991

Kopf AW, Gold RS, Rogers GS, Hennessey NP, Friedman RJ, Rigel DS, Levenstein M: Relationship of lumbosacral nevocytic nevi to sun exposure in dysplastic nevus syndrome. Arch Dermatol 122:1003–1006, 1986

Kopf AW, Lindsay AC, Rogers GS, Friedman RJ, Rigel DS, Levenstein M: Relationship of nevocytic nevi to sun exposure in dysplastic nevus syndrome. J Am Acad Dermatol 12:656–662, 1985

Meyer LJ, Goldgar DE, Cannon-Albright LA, Piepkorn MW, Zone JJ, Risman MB, Skolnick MH: Number, size, and histopathology of nevi in Utah kindreds. Cytogenet Cell Genet 59:167–169, 1992

Parry D, Peters G: Temperature-sensitive mutants of p16CDKN2 associated with familial melanoma. Mol Cell Biol 16:3844–3852, 1996

Searle AG: *Comparative Genetics of Coat Colour in Mammals*. New York: Academic Press Inc., 1968

Slade J, Marghoob AA, Salopek TG, Rigel DS, Kopf AW, Bart RS: Atypical mole syndrome: Risk factor for cutaneous malignant melanoma and implications for management. J Am Acad Dermatol 32:479–494, 1995

Webb P: Temperatures of skin, subcutaneous tissue, muscle and core in resting men in cold, comfortable and hot conditions. Eur J Appl Physiol Occup Physiol 64:471–476, 1992

Wenger CB: The regulation of body temperature. In: Rhodes RA, Tanner GA (eds). *Medical Physiology*. Boston: Little, Brown and Company, 1995; p 587–613