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A Quantitative Assessment of the Burden and Distribution of Lisch Nodules in Adults with Neurofibromatosis Type 1

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Abstract

Purpose—The presence of two or more Lisch nodules (melanocytic hamartomas of the iris) is one of seven diagnostic criteria for neurofibromatosis type 1 (NF1), a common monogenic disorder of dysregulated neurocutaneous growth. We investigated the hypothesis that Lisch nodules arise secondary to ultraviolet (UV) radiation exposure from sunlight.

Methods—We mapped and quantified Lisch nodule burden in the irides of 77 adults with NF1. We also inventoried lifetime sunlight (UV radiation) exposure, determined NF1 neurocutaneous severity, and selectively genotyped two *NF1* mutations predictive of severity.

Results—There was high inter-individual variability in Lisch nodule burden. Lisch nodules were primarily located in the inferior hemifield (half) of the iris, regardless of its color ($P = 3.0 \times 10^{-20}$). Light irides harbored significantly more Lisch nodules than dark irides ($P = 4.8 \times 10^{-5}$). There was no statistically significant correlation of Lisch nodule burden to lifetime sunlight exposure “dose” or NF1 neurocutaneous severity.

Conclusions—The difference in Lisch nodule burden between the superior and inferior iris hemifields is likely due to the sunlight-shielding effects on the superior half by peri-ocular structures. The difference in Lisch nodule burden between light and dark irides is likely due to the photo-protective effects of pigmentation. The genes underlying the control of iris color may thus be viewed as modifiers of severity of Lisch nodule burden in NF1. Given the role of UV radiation and, presumably, DNA damage in Lisch nodule pathogenesis, “benign tumor of the iris,” not “hamartoma,” may be a better descriptor.

Keywords

Neurofibromatosis type 1; Lisch nodule; Ultraviolet radiation; Iris color; Genetic modifiers

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Competing Interests

None

Introduction

Neurofibromatosis type 1 (NF1) is a common monogenic disorder of neurocutaneous tissue growth that arises secondary to mutations in the tumor suppressor gene *NF1*.^{1, 2} It features an autosomal dominant pattern of inheritance. Individuals with NF1 typically have an increased predisposition to a variety of benign and malignant tumors and develop neurofibromas, café-au-lait macules, axillary and inguinal freckling. Variable expressivity, even within families, is common in NF1 and is often attributed to genetic modifiers.^{3, 4} The *NF1* genotype does not predict severity, with the exception of a 3-bp inframe deletion in exon 17 (c.2970–2972 delAAT: absence of dermal neurofibromas)⁵ and microdeletion of *NF1* (high neurofibroma burden, dysmorphic features)⁶.

The NIH Consensus Criteria for the clinical diagnosis of NF1 include the presence of two or more Lisch nodules, traditionally described as “iris hamartomas.”⁷ They tend to appear early in life⁸ and only rarely in individuals without the disorder.⁹ Under slit-lamp magnification, Lisch nodules appear as well-defined, gelatinous, dome-shaped elevations of the iris surface. Aside from their usefulness in diagnosis, they have no known clinical consequence. It is controversial whether Lisch nodule burden predicts^{10, 11} or does not predict⁸ cutaneous severity in NF1. By adulthood, Lisch nodules are observed in over 90% of individuals with NF1.^{8, 10–16}

Little is known about the molecular pathogenesis of Lisch nodules. Two reports advocate a melanocytic origin of Lisch nodules.^{17, 18} A third study describes the presence of “pigmented cells, fibroblast-like cells and mast cells” in Lisch nodules in a pattern akin to a neurofibroma.¹⁹ The authors note that certain ultra-structural details in the pigmented cells and the presence of mast cells are evidence of a “Schwannian origin” of Lisch nodules.

Ultraviolet (UV) radiation from sunlight is hypothesized to be important in the pathogenesis of Lisch nodules.^{12, 20} The unequal distribution of Lisch nodules in the iris is hypothesized to be secondary to the relative shielding of the superior hemifield (half) of the iris from sunlight-derived UV radiation (hereafter, “UV radiation”) by the eyelid and eyebrow.^{12, 21}

The role of UV radiation in Lisch nodule pathogenesis is controversial²² and has not been formally evaluated. In this report, we quantitatively determined Lisch nodule burden (number of Lisch nodules in the irides) and distribution in a large cohort of adults with NF1. We hypothesized *a priori* that the inferior hemifield of the iris harbors a greater number of Lisch nodules than the superior one, due to the shielding effects of peri-ocular structures.^{23–25} Second, we examined the effects of iris color (dark vs. light) on Lisch nodule burden. Third, we collected quantitative estimates of ocular UV radiation exposure and explored its effects on Lisch nodule burden. Fourth, we investigated the relationship of Lisch nodule burden with café-au-lait macule and neurofibroma burden, two measures of NF1 neurocutaneous severity. Fifth, we examined differences in Lisch nodule burden between the left and right irides. Lastly, we determined the frequency of certain *NF1* genotypes (specifically, c.2970–2972 delAAT and *NF1* microdeletion) in our population and evaluated their effects, if any, on Lisch nodule burden.

Methods

Lisch Nodule Evaluation and Determination of NF1 Neurocutaneous Severity

In a natural history study of neurofibromatosis type 1, we evaluated 77 post-pubertal individuals from 56 families who met the NIH consensus criteria for NF1.⁷ Each individual was evaluated by a single physician (DRS). Both eyes were digitally photographed with a slit-lamp under consistent lighting conditions (ReSeeVit 5V, Veatch Ophthalmic Instruments,

Tempe, AZ). For each eye, a map of the distribution and number of Lisch nodules was sketched during the evaluation. Care was taken to note Wolfflin-Kruckman spots, iris mammillations and flat iris lesions such as freckles and nevi. During a history and physical examination, a semi-quantitative determination of the number dermal neurofibromas (0, 1–10, 11–100, 101–500, 500+) and a count and distribution of café-au-lait macules greater than 1.5 cm (using a Wood's lamp and ruler) was made. The study was approved by the institutional review board of the National Human Genome Research Institute and followed the tenets of the Declaration of Helsinki. After explanation of the nature and possible consequences of the study, informed consent was obtained from all participants.

Determination of Iris Color

The color of each iris was determined by comparing it to three photographic standards obtained from the Beaver Dam Eye study.²⁶ The three standards varied from little iris pigment (blue-grey) to significant iris pigment (medium to dark brown). We compared each iris to the two extreme standards to define it as “light” (blue-grey to hazel) or “dark” (brown). Three masked observers independently examined each iris with disagreements settled by consensus.

Mapping Lisch Nodule Distribution

We used NIH Image software (ImageJ v. 1.40 for Macintosh OS X, (<http://rsbweb.nih.gov/ij/index.html>)) to process the digital slit-lamp images. The pupil and corneal light reflection spots (if any) and surrounding cornea were removed, leaving solely the iris for analysis. The Walter bandpass filter (ImageJ plug-in: <http://rsb.info.nih.gov/ij/docs/source/ij/plugin/filter/FFTFILTER.java.html>) was then applied to the image to smooth and equalize the shading. Another ImageJ plug-in, “Threshold Colour” (<http://rsb.info.nih.gov/ij/plugins/rgb-measure-plus.html>) was then used to filter out all non-Lisch nodule color. The position of each Lisch nodule was verified with the map made at the time of slit-lamp photography. Nodule location (on a Cartesian plane, relative to the pupil center), area, distance from pupil center, and maximum nodule diameter were recorded. The resulting image was a map of Lisch nodule “silhouettes” and permitted precise assignment of each nodule into one of the four quadrants of each iris: inferonasal, inferotemporal, superonasal or superotemporal. The images were also compiled into stacks; using the “z-project” function in ImageJ, the stacks could be projected to render a collective map of Lisch nodule distribution and density. The color of the map was calibrated to reflect the density of Lisch nodules in a given area.

Quantification of Environmental UV Radiation Exposure

Participants completed a questionnaire that sought to quantify environmental UV radiation exposure (Supplementary Figure 1) using both quantitative measures (years) and five-point Likert scales (1 = “never”; 3 = “occasionally”; 5 = “frequently”). We measured both easily recalled objective data (e.g. age, places lived) and more subjective data (recall of frequency of sunglasses use and time spent outdoors). Specifically, we inventoried the length of time lived in various locales since birth (years), time spent wearing eyeglasses or contact lenses (years), frequency of sunglasses use in the last ten years (Likert scale), amount of time spent outdoors between 10 AM and 3 PM in the last ten years (Likert scale), amount of time spent outdoors in spring and summer (April-August) for work, home, and recreation (Likert scale) and any history of intense UV radiation-exposure activities such as spring skiing, ice fishing or over-water fishing.^{25, 27–32} From the list of places lived, we calculated a weighted lifetime average latitude and elevation. The percent of lifespan spent wearing eyeglasses and/or contact lenses was calculated; eyeglasses and contact lenses filter UV radiation and thus decrease exposure to ocular tissues. Sunglasses may paradoxically increase UV radiation exposure to ocular

tissues.²⁵ The five-point Likert scales for time spent outdoors for work, home and recreation were summed as the “Outdoor Score” (range 3–15).

Statistical Analysis and Control of Multiple Hypothesis Testing

Before collecting our data on Lisch nodule distribution, iris color and UV radiation exposure, we *a priori* formulated five primary and 33 secondary hypotheses about Lisch nodule distribution, pathogenesis and natural history (Tables 1 and 2). The prior formulation of primary hypotheses prevented the statistically deleterious effects of uncontrolled *a posteriori* data exploration. For each individual, we summed the number of Lisch nodules from the right and left irides. We used non-parametric tests (the signed rank and Kruskal-Wallis tests of location in paired and unpaired data, respectively and the Spearman rank correlation coefficient), which do not assume normality and are conservative.

For the five primary hypotheses, we used the Bonferroni principle to construct conservative approximate 95% simultaneous confidence intervals about the five primary estimated effects by erecting a 99% individual confidence interval about each one. To determine the confidence intervals, we used Fisher’s approximation with Pearson’s correlation coefficient (hypotheses numbers 4 and 5) and standard methods for paired differences (hypotheses number 1 and 3) and differences between groups (hypothesis number 2). These intervals allow us to reject, at an experiment-wise level of 5%, any set of hypothesized effect values that does not lie completely within the five 99% confidence intervals.

Our dataset is powered to detect strong to moderate effects in a correlation analysis. To determine power in our five primary and selected secondary hypotheses, we calculated that a true correlation of 0.39 or better was needed to guarantee at least 80% power (if $N > 70$, $\alpha = 0.01$, two-tailed). Additionally, for the comparison of Lisch nodules in nasal and temporal hemifields, we calculated that a true hemifield difference of least 2.7 nodules is needed; this assumes a standard deviation of 7, a value derived from our data.

To control for the effects of multiple hypothesis testing, we applied the conservative Bonferroni principle to the nominal *P*-values determined for the five primary hypotheses. The 33 secondary hypotheses are exploratory and investigate 1) the role of certain behaviors and geography (eyeglasses/sunglasses use, time spent outside, elevation) on Lisch nodule burden, 2) correlation of Lisch nodule burden with neurocutaneous severity (neurofibroma and café-au-lait macule burden), 3) Lisch nodule burden in certain iris quadrants and, 4) the role of gender in Lisch nodule burden. Since no multiple testing correction was applied caution is needed interpreting a “significant” secondary hypothesis.

We used the following model to distinguish contributions to Lisch nodule burden variance between patients from contributions between eyes within patients:

$$x(i, j) = \mu + \tau(i) + \varepsilon(i, j) \quad (\text{Equation 1})$$

where x = Lisch nodule burden for a given eye, $i = 1, \dots, n = 77$ people, $j = (1, 2)$ = (left eye, right eye), μ = overall mean Lisch nodule burden, $\text{var}(\tau)$ = inter-individual Lisch nodule variance, and $\text{var}(\varepsilon)$ = variance due either to eyes within individuals, or to stochastic noise. We modeled the percentage of Lisch nodule variance due to the person as $100 * \text{var}(\tau) / (\text{var}(\tau) + \text{var}(\varepsilon))$.

NF1 Genotyping: c.2970–2972 delAAT and NF1 Microdeletion

We sought evidence of the 3-bp inframe deletion in exon 17 (c.2970–2972 delAAT) in ten individuals with NF1 with no palpable dermal neurofibromas. Genotyping was performed as

per Upadhyaya *et al.*⁵ Briefly, a 386 base-pair fragment was amplified with forward (5'-ATTGGCTCTATGCCTGTGG-3') and reverse (5'-CACACCCTAGTTTGTGTGCAG-3') primers. Purified amplicons were sequenced with forward and reverse sequencing primers and analyzed with DNASTAR Lasergene software (DNASTAR, Madison, WI, USA). In 12 individuals from 12 families with high neurofibroma burden (500+), we sought evidence of the *NF1* microdeletion by assessment of loss of heterozygosity (LOH) by single nucleotide polymorphism (SNP) genotyping. We selected 19 TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) with a SNP minor allele frequency of ~0.25 or greater spanning ~1.5 Mb of the *NF1* locus (Table 3). To aid in haplotype construction, we genotyped all available family members from the 14 sample and control pedigrees (44 individuals). The reactions were performed with a 7500 Fast Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocol. Genotype data was assessed by Merlin software for mendelian inconsistencies.³³ Haplotypes of the *NF1* locus were constructed by hand and confirmed by Merlin. For those individuals with non-informative SNP genotyping, we performed multiplex ligation probe analysis (MLPA, Holland MRC, Amsterdam, The Netherlands) of the *NF1* locus according to the manufacturer's instructions.

Results

A Range of Age, Ethnicity, NF1 Neurocutaneous Severity, UV Radiation Exposure and Iris Color Was Observed

The 77 participants ranged in age from 15.5 years to 76 years with an average age of 37 years and a median age of 34 years. There were 28 males and 49 females and 18 parent-child pairs and 7 sibling pairs. Seven people noted African ancestry (9%) and two people noted Asian ancestry (2.6%). A spectrum of severity of the neurocutaneous manifestations of NF1 was represented. Accurate counts of café-au-lait macule burden in 76/77 (99%) participants were available and ranged from 4 to 34 café-au-macules greater than 1.5 cm (mean: 16, median 14). We assigned a semi-quantitative assessment of dermal neurofibroma burden to 71/77 (92%) of participants. Of the assessed 71 participants, 11% had no apparent dermal neurofibromas, 34% had 1–10 dermal neurofibromas, 23% had 11–50 dermal neurofibromas, 6% had 51–100 dermal neurofibromas, 11% had 101–500 dermal neurofibromas and 15% had 500 or more dermal neurofibromas.

We obtained completed UV radiation exposure questionnaires from 73/77 (95%) of the participants. Two females (one with dark irides, and one with light irides) were lost to follow-up. For two other participants (a brother and sister, both with light irides), we obtained data only on places lived from a family member. Participants spent the majority of their lives in the United States, a fact reflected in the range of weighted lifetime average latitude (20.7 to 47.6 degrees north) and elevation (sea level to > 5000 feet). For the 73 participants with completed questionnaires, 16 (22%) never wore eyeglasses or contact lenses. Of the remaining 78% of participants, eyeglasses/contact lenses use ranged from less than 1% to greater than 95% of lifespan. A wide range of frequency of sunglasses use in the last ten years (4 with no data, range 1 – 5, mean = 3.4), amount of time spent outdoors between 10 AM and 3 PM in the last ten years (4 with no data, range 1 – 5, mean = 3.76), Outdoor Scores and history of intense UV radiation exposure (5 with no data, range 3 – 15, mean = 9.09) activities were observed.

There were 49 (64%) participants with light irides (age range: 15.5 – 76 years; mean 38 years) and 28 (36%) participants with dark irides (age range: 16 – 59 years; mean 35 years). Of the 28 individuals with dark irides, 8 were of African or Asian ancestry. One man with African and Caucasian ancestry had light irides. There were no examples of heterochromia.

Unequal Distribution of Lisch Nodule Burden in Superior vs. Inferior Iris Hemifields But Not in Temporal vs. Nasal Iris Hemifields

Lisch nodules were observed in 73/77 (95%) of participants. All participants had clear images from both irides. Of the four individuals without observed Lisch nodules, three had dark irides (31-year-old female, 24-year-old male and 35-year-old-male) and one had light irides (48-year-old female). Of the five primary hypotheses subjected to correction for multiple testing (Table 1), number one (number of Lisch nodules in the inferior hemifield of the iris is greater than the number of nodules in the superior hemifield of the iris) was statistically significant (Bonferroni adjusted $P = 3.0 \times 10^{-20}$). Figure 1 maps the distribution of 2,826 Lisch nodules from both irides of all participants. To investigate the Corneo effect (focusing of UV radiation on the medial limbus by the cornea³⁰), we examined differences in the quantity of Lisch nodules in temporal and nasal hemifields (hypothesis number three) but found no statistically significant evidence of unequal distribution. An unequal distribution of Lisch nodules was observed between the superonasal and superotemporal quadrants (Table 2, secondary hypothesis number 14a, signed rank test nominal $P = 0.02$). An unequal distribution of Lisch nodules between the superior quadrants is also evident in Figure 1. The distribution of Lisch nodules between the inferonasal and inferotemporal quadrants (Table 2, secondary hypothesis number 14b) was of borderline significance (signed rank test nominal P -value = 0.02), but in the *opposite* direction). However, since tests of secondary hypotheses were not *a priori* subject to Bonferroni correction, their significance, if any, must be interpreted with caution.

Light Irises Harbor Significantly More Lisch Nodules than Dark Irises

Hypothesis number two (light irides harbor a greater number of Lisch nodules than individuals with dark irides) was statistically significant (Bonferroni corrected $P = 4.8 \times 10^{-5}$). The mean number of Lisch nodules in individuals with dark and light irides was 8.6 and 52.8, respectively (2-sample t-test $P < 0.0001$). The median number of Lisch nodules in individuals with dark and light irides was 4 and 35, respectively (Kruskal-Wallis $P = 0.0002$). Figures 2 and 3 map the distribution of Lisch nodules from both eyes of participants with dark and light irides, respectively. In both light and dark irides, the number of Lisch nodules in the inferior hemifield of the iris was significantly greater than the number of nodules in the superior hemifield of the iris (Bonferroni corrected $P = 0.004$ (dark irides); Bonferroni corrected $P < 0.0001$ (light irides)).

Lisch Nodule Burden Does Not Significantly Correlate with Age, Weighted Lifetime Latitude of Residence or Other Measures of Differential UV Radiation Exposure

The two primary hypotheses investigating Lisch nodule burden and differential amounts of UV radiation exposure in our sample (Table 1, hypothesis numbers four and five: southerly latitudes and increasing age) were not significant. Nearly all of the secondary hypotheses (Table 2) exploring the relationship of Lisch nodule burden and age and various measures of UV radiation exposure, even after adjustment for iris color, were non-significant. One of the secondary hypotheses had marginally significant nominal P -values (number 9c: Individuals with light irides and greater time spent outdoors between 10 AM and 2 PM will have more Lisch nodules; nominal Pearson P -value: 0.06, nominal Spearman P -value: 0.02). However, after Bonferroni correction this hypotheses is not significant.

Lisch Nodule Burden Not Significantly Correlated with Neurofibroma or Café-au-Lait Macule Burden

The secondary hypotheses exploring the correlation of café-au-lait macule burden (numbers 11a, b, c) and dermal neurofibroma burden (numbers 12a, b, c) with Lisch nodule burden were non-significant (Table 2). Lisch nodule burden in the eight participants who lacked palpable dermal neurofibromas varied, depending on iris color: in the three individuals with dark irides,

Lisch nodule burden ranged from 1 to 4; in the four individuals with light irides, the burden ranged from 7 to 64. Similarly, of the eleven participants with an estimated 500 or more dermal neurofibromas, five had dark irides; the Lisch nodule burden in these individuals ranged from zero to 20 Lisch nodules. In the six individuals with light irides, the burden ranged from 16 to 167 Lisch nodules. In participants at the extremes of dermal neurofibroma burden, Lisch nodule burden was primarily determined by iris color.

Lisch Nodule Burden in Left Iris Predicts Burden in Right Iris

To investigate the degree to which Lisch nodule burden in one iris predicts Lisch nodule burden in the contra-lateral iris, we determined the Pearson and Spearman correlation coefficients. The Pearson correlation between left iris and right iris was 0.96 ($P < 0.0001$) and the Spearman correlation was 0.91 ($P < 0.0001$). The mean number of Lisch nodules in the right and left irides was 18.4 and 18.3, respectively ($P =$ not significant). Lisch nodule burden in the left iris is plotted against burden in the right iris in Figure 4. To understand the contribution of inter-individual variance and between-iris variance in the determination of Lisch nodule burden, we estimated these values to be: $\text{Var}(\tau) = 608.8$ and $\text{Var}(\epsilon) = 28.8$. That is, about 95% of the total variance of Lisch burden is due to differences between individuals; Lisch nodule burden between irides differs little within an individual.

The *NF1* c.2970–2972 delAAT Mutation and Microdeletion Were Rarely Observed in Our Population

Only one individual of the ten samples sequenced for the *NF1* c.2970–2972 delAAT mutation harbored the mutation. The participant, a 44-year-old female with hazel (light) irides, had a total of 11 Lisch nodules in her two eyes (30th centile for Lisch nodule burden among individuals with light irides). In this one individual, the *NF1* c.2970–2972 delAAT mutation does not preclude Lisch nodule development.

In five individuals, all or nearly all the genotyped *NF1* SNP loci were heterozygous; an *NF1* microdeletion was deemed unlikely. The remaining seven samples were non-informative at all or nearly all the genotyped *NF1* SNP loci. In these seven samples plus a control and an additional sample not subject to SNP genotyping, a microdeletion of the *NF1* gene was excluded by multiplex ligation probe analysis MLPA.

Discussion

Our data show that the majority of Lisch nodules are located in the inferior hemifield of the iris, regardless of its color. This is consistent with the effects of differential UV radiation exposure. Second, we showed that light irides harbor significantly more Lisch nodules than dark irides. Third, for modest effect sizes and regardless of iris color we did not find evidence of significant correlation between Lisch nodule burden and two measures of neurocutaneous severity in *NF1*, café-au-lait burden and neurofibroma burden. This is in contrast to earlier reports^{10, 11} which did not control for iris color. Fourth, for modest effect sizes and regardless of iris color we observed no statistically significant correlation between Lisch nodule burden and various measures of UV radiation exposure. Fifth, the one example of the *NF1* c.2970–2972 delAAT mutation in our population was not associated with an absence of Lisch nodules. Lastly, Lisch nodule burden in one iris is highly predictive of Lisch nodule burden in the contra-lateral iris. An analysis of variance components found high inter-individual, but not between-eye, variance in Lisch nodule burden in our population. Taken together, our data suggest that *NF1*-haploinsufficiency and ocular UV radiation exposure are, in most individuals, necessary and sufficient for the pathogenesis of Lisch nodules. However, the ultimate burden of Lisch nodules is modulated by other non-random factors.

The most important of these factors is iris color, which in humans is primarily determined by the quantity and ratio of eumelanin (dark brown-to-black in color) and pheomelanin (reddish-pink in color).^{34–36} Melanins protect against the adverse effects of UV radiation by serving as a physical photo-screen and as a free radical scavenger. Melanins are produced by the melanocytes of the iris stroma from which Lisch nodules are thought to arise.^{17, 18} The antioxidant properties of eumelanin are superior to those of pheomelanin.³⁵ Upon exposure to UV radiation, pheomelanin can generate hydroxyl radicals and superoxide anions and thus may be photo-toxic itself.³⁶ Consistent with this observation, individuals with light irides are at greater risk for uveal melanoma, which like Lisch nodules also has an inferior hemifield predilection.^{35, 37}

To investigate the high inter-individual variability ($\text{var}(\tau)$) in Lisch nodule burden, we examined the role of two important *NF1* genotypes and lifetime UV radiation exposure. The *NF1* c.2970–2972 delAAT mutation and *NF1* microdeletion were rare in our population; the single example of the Δ AAT mutation did not preclude Lisch nodule development. Quantification of any environmental exposure, such as UV radiation from sunlight, is difficult and is limited by participant recall. History of residence (lifetime weighted latitude) and participant age, both easily recalled, have a direct correlation with lifetime UV radiation exposure.³² However, in our study, neither measure correlated with Lisch nodule burden. It is possible that local geographic and atmospheric factors (e.g. ground cover, cloud cover, stratospheric ozone) affecting albedo exert a greater influence on ocular UV radiation exposure than participant age and lifetime average latitude.³⁰ Such factors were not inventoried in our survey. It is also possible that Lisch nodule pathogenesis is akin to skin melanoma where dose per exposure, not cumulative UV radiation dose, is critical.³⁶ We asked about a history of spring skiing, ice fishing and over-water fishing, behaviors known to generate intense UV radiation exposures.³² No correlation with Lisch nodule burden and these activities was observed, although the number of participants was small. Lastly, the lack of correlation between an environmental exposure and Lisch nodule burden may reflect the limitations of our questionnaire, which has not been validated to gauge its efficacy in determining lifetime UV radiation exposure.

In skin melanocytes, there is a *dose-dependent* generation of cyclobutane pyrimidine dimers after exposure to UV radiation.³⁶ However, there is wide *inter-individual* variability in the rate of DNA repair following the minimal erythema dose of UVA/UVB radiation to skin, even after adjustment for skin photo-type.³⁸ Bi-allelic inactivation of *NF1* is observed in many *NF1*-associated tumors,^{39–41} suggesting that DNA damage and repair are central in the pathogenesis of *NF1*. Bi-allelic inactivation of *NF1* has also been described in melanocytes from café-au-lait macules from two individuals with mosaic *NF1*.⁴²

We are not aware of any investigation demonstrating *NF1* bi-allelic inactivation in Lisch nodules. However, *NF1* bi-allelic inactivation in melanocytes secondary to UV radiation-induced DNA damage is a plausible, even likely, mechanism for Lisch nodule pathogenesis. Lisch nodules are usually described as “iris hamartomas.” The term “hamartoma” typically applies to an excessive but focal overgrowth of cells and tissues native to the organ in which it occurs. Although the cellular elements are mature, they do not reproduce the normal architecture of the surrounding tissue. They are frequently seen in infancy and childhood.⁴³ The use of this term should be reconsidered given the histologic and ultra-structural evidence, in a recent report, of fibroblast-like cells and mast cells, in addition to pigmented cells, in Lisch nodules.¹⁹ The presence of fibroblast-like cells and mast cells, absent in common melanocytic nevi but observed in neurofibromas, suggests that Lisch nodules are more akin to benign tumors like neurofibromas than hamartomas.¹⁹ These pathologic observations, if replicated, combined with evidence of an increase in Lisch nodule burden in adult life, and not just in childhood, would also be consistent with the behavior of an *NF1*-associated benign tumor. Given the

pathologic evidence, the role of UV radiation and, presumably, DNA damage in their pathogenesis, “benign tumor of the iris” may be a more appropriate description for Lisch nodules.

The elevated inter-individual variance ($\text{var}(\tau)$), low between-eye and stochastic variance ($\text{var}(\epsilon)$) and absence of a UV radiation dose effect highlight the importance of individual, possibly genetic, differences in the determination of Lisch nodule burden. The lack of significant correlation between neurofibroma burden and café-au-lait burden with Lisch nodule burden is consistent with previous studies advocating the existence of trait-specific modifying loci in NF1.^{3, 4} Genetic modifiers may explain 1) the high correlation in Lisch nodule burden between eyes, 2) the variability in age of appearance of Lisch nodules,⁸ 3) the range in burden for a given iris color and 4) the lack, in multiple studies including this one, of Lisch nodules in 5–10% of adults with NF1. Genetic modifiers of Lisch nodule burden might 1) influence the efficiency of response to UV radiation-inflicted DNA damage, 2) buffer reactive oxygen species, and/or 3) direct DNA repair. Lastly, iris color itself is hereditary⁴⁴ and is of primary importance in the determination of Lisch nodule burden. The genes influencing iris color may themselves be reasonably viewed as genetic modifiers of severity of Lisch nodule burden in NF1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Map of Lisch nodule distribution in the right and left irides. Data are from 73 adults with NF1 and Lisch nodules. Data from both dark and light irides are included. Nodules are displayed as silhouettes of their digital images.

Figure 2.

Map of Lisch nodule distribution in the right and left irides. Data are from 25 individuals with NF1 and Lisch nodules who also have dark irides. Nodules are displayed as silhouettes of their digital images.

Figure 3. Map of Lisch nodule distribution in the right and left irides. Data are from 48 individuals with NF1 and Lisch nodules who also have light irides are included. Nodules are displayed as silhouettes of their digital images.

Correlation of Lisch Nodule Burden: Left vs. Right Eyes Includes Both Dark and Light Irides

Figure 4.
Lisch nodule burden in left iris is highly correlated with burden in right iris.

Table 1

Primary hypotheses investigating the relationship of sunlight-derived UV radiation exposure and Lisch nodule burden in individuals with NF1. Parametric tests (paired t-test, 2-sample t-test and Pearson correlation coefficient) were also performed as confirmation (data not shown). N.S. = not significant

Number	Hypothesis	Dataset	Test	P-Value	Bonferroni P-Value	Confidence Interval (95% Simultaneous)
1	The number of Lisch nodules in the inferior hemi-field of the iris is greater than the number of nodules in the superior hemi-field of the iris.	All individuals (N = 77)	Signed rank	6.1×10^{-21}	3.04×10^{-20}	10.79 – 22.82
2	Individuals with light colored irides harbor a greater number of Lisch nodules than individuals with dark irides.	Dark irides: N = 28 Light irides: N = 49	Kruskal-Wallis	9.6×10^{-6}	4.8×10^{-5}	-66.16 – -22.14
3	The number of Lisch nodules in the nasal hemi-field of the iris is less than the number of nodules in the temporal hemi-field. (See also hypotheses #14a and 14b)	All individuals (N = 77)	Signed rank	N.S.	N.S.	-2.34 – 1.88
4	Individuals who have a more southerly weighted latitude of residence have more Lisch nodules. (See also hypotheses #15a and 15b)	All individuals with latitude data (N = 75)	Spearman correlation	N.S.	N.S.	-0.27 – 0.32
5	Lisch nodule burden will increase with age. (See also hypotheses 16a and 16b)	All individuals (N = 77)	Spearman correlation	N.S.	N.S.	-0.28 – 0.30

Table 2

Secondary hypotheses investigating the role of certain behaviors and geography on Lisch nodule burden, correlation of Lisch nodule burden with neurocutaneous severity, Lisch nodule burden in certain iris quadrants and gender in individuals with NF1. Parametric tests (paired t-test, 2-sample t-test and Pearson correlation coefficient) were also performed as confirmation (data not shown). N.S. = not significant

Number	Hypothesis	Dataset	Test	P-Value
6a	Individuals who have a greater weighted elevation of residence will have more Lisch nodules.	All individuals with elevation data (N = 74)	Spearman correlation	N.S.
6b	Individuals with dark irides who have a greater weighted elevation of residence will have more Lisch nodules.	All individuals with elevation data and dark irides (N = 26)	Spearman correlation	N.S.
6c	Individuals with light irides who have a greater weighted elevation of residence will have more Lisch nodules.	All individuals with elevation data and light irides (N = 48)	Spearman correlation	N.S.
7a	Individuals with a greater percentage of life spent wearing eyeglasses or contact lenses will have fewer Lisch nodules.	All individuals with eyeglass/contact lenses data (N = 73)	Spearman correlation	N.S.
7b	Individuals with dark irides and a greater percentage of life spent wearing eyeglasses or contact lenses will have fewer Lisch nodules.	All individuals with eyeglass/contact lenses data (N = 27)	Spearman correlation	N.S.
7c	Individuals with light irides and a greater percentage of life spent wearing eyeglasses or contact lenses will have fewer Lisch nodules.	All individuals with eyeglass/contact lenses data (N = 46)	Spearman correlation	N.S.
8a	Individuals with more frequent sunglasses use will have fewer Lisch nodules.	All individuals with sunglasses data (N = 73)	Spearman correlation	N.S.
8b	Individuals with dark irides and more frequent sunglasses use will have fewer Lisch nodules.	All individuals with sunglasses data (N = 27)	Spearman correlation	N.S.
8c	Individuals with light irides and more frequent sunglasses use will have fewer Lisch nodules.	All individuals with sunglasses data (N = 46)	Spearman correlation	N.S.
9a	Individuals with greater time spent outdoors between 10 AM and 2 PM will have more Lisch nodules.	All individuals with time outside data (N = 73)	Spearman correlation	N.S.
9b	Individuals with dark irides and greater time spent outdoors between 10 AM and 2 PM will have more Lisch nodules.	All individuals with time outside data (N = 27)	Spearman correlation	N.S.
9c	Individuals with light irides and greater time spent outdoors between 10 AM and 2 PM will have more Lisch nodules.	All individuals with time outside data (N = 46)	Spearman correlation	0.02
10a	Individuals with a higher cumulative Outdoor Score will have more Lisch nodules.	All individuals with Outdoor Score data (N = 72)	Spearman correlation	N.S.
10b	Individuals with dark irides and a higher cumulative Outdoor Score will have more Lisch nodules.	All individuals with Outdoor Score data (N = 27)	Spearman correlation	N.S.
10c	Individuals with light irides and a higher cumulative Outdoor Score will have more Lisch nodules.	All individuals with Outdoor Score data (N = 45)	Spearman correlation	N.S.
11a	Lisch nodule burden will positively correlate with severity of café-au-lait macule burden	All individuals with CALM burden data (N = 76)	Spearman correlation	N.S.
11b	In individuals with dark irides, Lisch nodule burden will positively correlate with severity of café-au-lait macule burden	All individuals with CALM burden data (N = 27)	Spearman correlation	N.S.
11c	In individuals with light irides, Lisch nodule burden will positively correlate with severity of café-au-lait macule burden	All individuals with CALM burden data (N = 48)	Spearman correlation	N.S.

Number	Hypothesis	Dataset	Test	P-Value
12a	Lisch nodule burden will positively correlate with severity of neurofibroma burden	All individuals with cutaneous neurofibroma burden data (N = 71)	Spearman correlation	N.S.
12b	In individuals with dark irides, Lisch nodule burden will correlate with severity of neurofibroma burden	All individuals with cutaneous neurofibroma burden data (N = 25)	Spearman correlation	N.S.
12c	In individuals with light irides, Lisch nodule burden will correlate with severity of neurofibroma burden	All individuals with cutaneous neurofibroma burden data (N = 43)	Spearman correlation	0.08
13	Lisch nodule burden will correlate with a history of ever participating in intense UV-exposure activities. <i>Specific activities:</i>			
13a	<i>Spring snow skiing</i>	All individuals with activity data (N = 72)	Kruskal-Wallis	N.S.
13b	<i>Ice fishing</i>	All individuals with activity data (N = 72)	Kruskal-Wallis	N.S.
13c	<i>Ocean, bay or lake fishing</i>	All individuals with activity data (N = 72)	Kruskal-Wallis	N.S.
14a	The number of Lisch nodules in the superior nasal hemi-field of the iris is less than the number of nodules in the superior temporal hemi-field.	All individuals (N = 77)	Signed rank	0.02
14b	The number of Lisch nodules in the inferior nasal hemi-field of the iris is less than the number of nodules in the inferior temporal hemi-field.	All individuals (N = 77)	Signed rank	0.02 (t = -1.83)
15a	Individuals with dark irides who have a more southerly weighted latitude of residence have more Lisch nodules.	All individuals with latitude data and dark irides (N = 27)	Spearman correlation	N.S.
15b	Individuals with light irides who have a more southerly weighted latitude of residence have more Lisch nodules.	All individuals with latitude data and dark irides (N = 48)	Spearman correlation	N.S.
16a	Lisch nodule burden will increase with age in individuals with dark irides	All individuals with dark irides (N = 28)	Spearman correlation	N.S.
16b	Lisch nodule burden will increase with age in individuals with light irides	All individuals with dark irides (N = 49)	Spearman correlation	N.S.
17a	Median Lisch nodule burden is not influenced by gender.	All individuals (N = 77)	Kruskal-Wallis	N.S.
17b	In individuals with light irides, median Lisch nodule burden is not influenced by gender.	All individuals with light irides (N = 28)	Kruskal-Wallis	N.S.
17c	In individuals with dark irides, median Lisch nodule burden is not influenced by gender.	All individuals with dark irides (N = 49)	Kruskal-Wallis	N.S.

Table 3

List of genotyped SNP loci used in the determination of *NF1* microdeletion status. Chromosome coordinates from dbSNP Build 129.

dbSNP Name	ABI Assay Name	Gene Symbol	Chr. 17 Base Position	Distance to Upstream SNP (bp)
rs7219456	C___403750_10	<i>EFCAB5</i>	25,398,238	0
rs8072345	C___7911284_10	<i>BLMH</i>	25,628,415	230,177
rs9913237	C___2980234_10	<i>CPD</i>	25,785,065	156,650
rs170053	C___1083154_10	<i>TBC1D29</i>	25,917,209	132,144
rs1123232	C___11619935_10	<i>NF1</i>	26,448,115	530,906
rs2012581	C___2263017_10	<i>NF1</i>	26,584,926	136,811
rs4795587	C___32294423_10	<i>NF1</i>	26,612,043	27,117
rs8080679	C___2193426_10	<i>NF1</i>	26,626,736	14,693
rs4795593	C___2193435_10	<i>NF1</i>	26,637,726	10,990
rs9902893	C___2533273_10	<i>NF1</i>	26,649,764	12,038
rs2040792	C___11941361_10	<i>NF1</i>	26,652,675	2,911
rs7505	C___7562823_1	<i>NF1</i>	26,668,978	16,303
rs7226006	C___2533298_10	<i>NF1</i>	26,674,782	5,804
rs2057769	C___11941321_10	<i>NF1</i>	26,715,494	40,712
rs1800845	C___7562857_10	<i>NF1</i>	26,727,564	12,070
rs178858	C___1083716_10	<i>RAB11FIP4</i>	26,797,877	70,313
rs11657523	C___2181826_10	<i>RAB11FIP4</i>	26,838,188	40,311
rs12937238	C___2181841_10	<i>RAB11FIP4</i>	26,846,982	8,794
rs2343245	C___16210542_10	<i>RAB11FIP4</i>	26,859,343	12,361