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A novel iris transillumination grading scale allowing flexible assessment with quantitative image analysis and visual matching

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Abstract

Purpose—To develop a sensitive scale of iris transillumination suitable for clinical and research use, with the capability of using image analysis of photographs or visual matching to a standard image set.

Design—Evaluation of Diagnostic Technology

Participants—Seventy study subjects with ocular or oculocutaneous albinism and a broad range of ocular hypopigmentation.

Methods—Iris transillumination photographic images were taken from existing albinism-related studies. A subset of high-quality images was subjected to computational image analysis and to ranking by both expert and non-expert reviewers. Ordering by a specific image analysis metric was compared with ordering by visual ranking. To establish an eight-point review scale, images were binned based on a specific image analysis metric.

Main Outcome Measures—Pairwise ranking consistency was evaluated by Spearman's rank correlation coefficient. Within-technique visual ranking results were compared with Kendall's coefficient of concordance analysis.

Results—There was a high degree of correlation among the image analysis, expert-based and non-expert-based image rankings. Pairwise comparisons of the quantitative ranking with each reviewer generated an average correlation coefficient (Kendall tau) of 0.83 +/- 0.04 (s.d.). Inter-rater correlation was also high with Kendall's W values of 0.96, 0.95 and 0.95 for non-expert, expert, and all reviewers respectively.

*First and second authors contributed equally to this work, as did penultimate and last authors.

Conclusions—The current standard for assessing iris transillumination is expert review of photographic or clinical exam findings. We adapted a straightforward image-analysis technique to generate quantitative transillumination values. Ranking by the quantitative values was shown to be highly similar to a ranking produced by both expert and non-expert reviewers. This finding suggests that the image characteristics used to quantify iris transillumination are not so subtle as to require expert interpretation. Inter-rater rankings were also highly similar, suggesting that varied methods of transillumination ranking are robust in terms of producing reproducible results.

INTRODUCTION

Albinism is caused by a group of genetic disorders associated with reduced melanin pigment biosynthesis. Oculocutaneous albinism (OCA) results in decreased pigmentation in the hair, skin and eyes. Most OCA is autosomal recessive and caused by one of four genes: *TYR* (OCA-1A and OCA-1B, MIM 203100), *OCA2* (OCA-2, MIM 203200), *TYRP1* (OCA-3, MIM 203290) and *SLC45A2* (OCA-4, MIM 606574). Additional rare OCA genes have recently been reported^{1–3}. A separate group of rare conditions combine OCA-like albinism and involvement of non-pigmented tissues. Examples include the Hermansky-Pudlak Syndrome (types 1 – 9) and Chediak Higashi Syndrome. Ocular albinism (*GPR143*, MIM 300500) is characterized by X-linked inheritance and hypopigmentation restricted principally to the eye. All types of OCA, as well as OA, are associated with variable nystagmus, foveal hypoplasia, retinal hypopigmentation, abnormal retinal axonal fiber decussation, and iris hypopigmentation. OCA has a substantial breadth of phenotypic heterogeneity confounding subtype diagnosis through phenotype evaluation⁴. To date, treatment of albinism is largely supportive, including low-vision aids, correction of refractive errors, treatment of amblyopia, and extraocular muscle surgery for strabismus and/or anomalous head posture.

Recent advances offer promise for new treatments for albinism. Gene replacement therapy has been successful in two rodent models of albinism—OCA-1 (caused by *TYR* mutations) and OA.^{5, 6} Based partly on observations by Lopez et al., a clinical trial of oral L-DOPA is currently underway (Clinical trial ID NCT01176435).⁷ In addition, our group has recently shown that nitisinone, a small molecule used in the treatment of Hereditary Tyrosinemia, type 1, improves ocular and fur pigmentation in a mouse model of OCA.⁸

Low vision in OCA is principally caused by a developmental eye defect. Therefore, an ideal treatment would start in young children in whom retinal development is not complete. Given safety considerations, however, pilot clinical trials will likely begin with adults. Because the adult visual system is largely mature, traditional vision-based outcome variables are unlikely to be changed by treatment. This observation has led us to consider ocular melanin content as an alternative measure of therapeutic success in proof-of-principle experiments. Iris transillumination is readily demonstrable under slit lamp examination and represents a reasonable indicator of iris melanin content. We are currently planning a one-year pilot clinical trial of nitisinone in patients with OCA-1B (i.e. partial tyrosinase deficiency), and need a fine-grained, preferably quantitative means of assessing iris transillumination. We have adapted a method developed in rodents⁹ and applied it to human subjects. We have also

designed an eight-point iris transillumination grading scale (ITGS) suitable for use in a clinical setting.

PATIENTS & METHODS

We reviewed the hospital records of albinism patients seen at the National Eye Institute's clinic from July 2007 to June 2012. Patients were evaluated under NIH-sponsored IRB-approved clinical research protocols 2009-HG-0035, 95-HG-0193, and T-EI-1033 in compliance with the Declaration of Helsinki and other relevant standards. Albinism was diagnosed on the basis of classic ocular and systemic signs by NEI ophthalmologists and/or NHGRI clinical geneticists. Iris transillumination was photographed before pupillary dilation on Haag-Streit BX 900 photo biomicroscope (10× magnification, 2–3 mm diameter of illumination beam) and captured on Cannon EOS 200. At least three images were taken for each patient. The photo quality was deemed acceptable if the illuminating beam was placed close to the center of the pupil and the size of the beam was close to that of the pupil. Patients displayed highly symmetrical ocular phenotype¹⁰; thus, which eye was analyzed was not controlled for in this study. Each transillumination image was saved as a 4.7 Mb uncompressed image file in a patient database.

Image analysis made use of Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA). One best image (based on uniformity and centrality of the corneal light reflex) was selected for each study participant. Each analyzed image was divided into four quadrants with vertical and horizontal lines transecting the center of the pupil. Using the elliptical marquee tool, a circle with diameter approximately 1/8th the diameter of the iris was drawn in the center of each quadrant. Gaussian blur with radius of 50 was applied to the area enclosed in the four circles. With the dropper tool, the red component of the RGB color within each circle was recorded. The values from the four circles were averaged to yield a quantitative transillumination (QTI) score for each image. Additional methods, including selection of the entire iris and the use of 30 circles instead of four, were tested and found to produce near-identical results. Therefore, the most straightforward method was adopted as our standard method.

Clinical Scoring tool

The possible range of QTI scores (0 – 255) was distributed into bins as follows. Scores of 0 – 15 were assigned as a “bin 0”--in essence no apparent iris transillumination. The remaining 240 points were divided into eight equal bins (1 – 8), with the highest number corresponding to the largest amount of apparent transillumination. The QTI bin boundaries and representative image counts from the initial cohort are shown in table 1. Three representative images were selected from each bin based on visual inspection. In some cases, additional images, not included in the original analysis, were added to complete a set of three high-quality images. Representative images are shown in Figure 1. The full scale is provided in high resolution in the supplemental materials.

Comparison of Visual Acuity to Transillumination

Depending on age, best-corrected visual acuity was measured using either an Early Treatment for Diabetic Retinopathy Study (ETDRS) chart or a Snellen projector chart. The number of letters read was converted to Snellen equivalent.

Comparison of QTI Scores to Visual Ranking

The *de facto* gold standard for evaluating iris transillumination is visual inspection at the time of ophthalmologic examination. Given the impracticality of arranging for multiple-observer evaluations of each patient, we used the inspection of transillumination photographs to order our cohort from least to greatest transillumination. Sixty-four iris transillumination images were printed in color at high resolution (1200×1200 dpi) as 3.5 × 5 inch cards. Seven volunteers with no ophthalmologic training and three ophthalmologists were asked to arrange the images in order from lightest (most iris transillumination) to darkest (no transillumination). The ranking generated by each evaluator was compared with the QTI score generated previously for each image.

Statistical Analysis

Some images had visually indistinguishable levels of iris transillumination, particularly those with no apparent deficit of iris pigmentation. Therefore, the resulting visual rankings contained non-unique ranks. To account for this data characteristic, Kendall's *tau* was used to compare each rater's rankings with image ranking by QTI score. Inter-rater ranking comparisons were carried out using Kendall's W, with correction for duplicate scores. All analyses were carried out using the R programming language. The Kendall's W analysis utilized the IRR package written by Matthias Gamer and colleagues. Prism Graphpad software (La Jolla, CA) was used for Spearman correlation coefficient calculation for QTI versus visual acuity.

RESULTS

Iris Transillumination Quantification (QTI Score)

Sixty-four images, each from a different individual, were judged to have adequate quality for transillumination assessment. QTI scores ranged from 1.0 to 228.3 (raw data available in Table S1). The QTI scores were not distributed uniformly across our cohort; the highest degrees of transillumination were underrepresented compared with the middle and lower degrees.

In order to ensure that the four areas chosen for quantification in each image were, in fact, representative of the whole, we analyzed 30 points at a fixed mid-iris radius in a random sample of 10 patients. The resulting transillumination values of the 4-quadrant and 30-point sampling method on average differed by 6.7% +/- 4.1% in the sampled images, suggesting a modest contribution of sample-site selection to the final QTI score.

Comparison of QTI Score with Visual Inspection

QTI-score based rankings were highly similar to those produced by visual inspection. There was a high degree of correlation between the QTI-score-based, expert-based and non-expert-

based image rankings. Pairwise comparisons of the quantitative ranking with each reviewer generated an average correlation coefficient (Kendall tau) of 0.83 ± 0.04 . Table 2 lists the statistical correlation of the individual pairwise comparisons. Inter-rater correlation was also high with a Kendall's W values of 0.96 (p-value 4.4×10^{-55}), 0.95 (p-value 3.9×10^{-13}) and 0.95 (p-value 1.7×10^{-99}) for non-expert, expert, and all reviewers respectively.

Correlation between Visual Acuity and Iris Transillumination

Our primary motivation for developing a fine-grained transillumination quantitation system was for use to measure iris transillumination in a research setting. However, such grading may ultimately prove useful for other purposes, including clinical application. Studies of children with albinism suggest that some ocular characteristics may have a modest prognostic value with regard to future visual acuity.¹¹

Among those characteristics is melanin pigment in the macula. We compared iris transillumination with visual acuity in 56 (83%) of our transillumination cohort for whom Snellen's measure of visual acuity data was available. The Spearman correlation coefficient (*rho*) between visual acuity and iris transillumination was modest: 0.4643 (95% C.I. 0.2140 – 0.6573). The correlation was, nonetheless, statistically significant with a p-value of 0.0005. Figure 2 shows visual acuity plotted against QTI score.

DISCUSSION

Herein we report a method to objectively evaluate albinism phenotypes through quantification of iris transillumination. We anticipate that this method will be of use in future clinical trials where a fine-grained quantitative method for evaluating iris transillumination will be necessary. Quantification of iris pigmentation has some advantages over assessment of melanin pigmentation in hair and skin. It is non-invasive, and the iris is neither subject to weathering/dyeing, nor UV inducibility. Furthermore, the method we have developed allows quantification by image analysis, a potential advantage for applications with precision requirements.

This study also expanded the current four-point iris transillumination grading scale¹² with the development of an eight-point scale. The eight-point scale offers clinicians a grading option with a higher degree of precision in documenting clinically-observed transillumination. Summers et al¹² graded iris transillumination on a four-point photo scale and found that, as a group, the children with greater iris pigmentation had a better prognosis with regard to future visual acuity. More recent studies^{10, 13} suggest a positive correlation between iris pigmentation and visual acuity, also supporting a prognostic role in early childhood. However, such studies, like our own, have shown modest correlation. Whether or not the QTI score will have real clinical utility likely depends on future work and the progress of current research into pro-melanogenic pharmacotherapy.

Our study must be interpreted in light of several important limitations. Firstly, the image analysis procedure we describe requires high quality iris transillumination photography. This can be challenging for younger children and persons with particularly severe photosensitivity and/or nystagmus. Optimally, the circumference of the illuminating beam

will precisely match that of the pupil, forming two concentric overlapping circles. If the illuminating beam deviates too far from the center of the pupil or is significantly larger or smaller than the pupil, the image will probably not be useful for QTI analysis. Among the seventy albinism medical records we reviewed, 64 (91%) had at least one transillumination image of good quality. In addition to the inherent variances in local iris transillumination, an off-centered illumination beam in the round pupil introduces hemispherical variances, from left to right, top to bottom, or diagonally, depending on the position of the beam. The four-point sampling method, one in each quadrant, appears to adequately average out some of these variances. This is demonstrated in the comparison of the four-point sampling method with the 30-point method.

The full validation of the 8-point transillumination scale was beyond the scope of the work for this publication. However, we plan to incorporate the evaluation into our future clinical albinism assessments to address important outstanding questions. These include the reproducibility of measurements within individuals, between photographers, and over time. Further, larger cohorts of evaluated study participants will be needed to see if the arbitrary, linear scaling model we used for the 8-point scale is optimal, versus a different scale function that better separates the intermediate transillumination values measured for the majority of our initial cohort.

In summary, we present a semi-quantitative scale for grading iris transillumination that will likely be useful in future clinical trials where a precise metric of iris pigmentation is required.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.
Single Representative Images of 8-Point Transillumination Scale

FIGURE 2.

Moderate correlation between visual acuity and iris transillumination with $r_s=0.4079$ and two tailed P value of 0.0024. However, a wide range of overlapping visual acuities exists with different degrees of iris transillumination.

Table 1

Convenience 8-Point Transillumination Scale

Scale score	Lower Bound Transillumination Score	Upper Bound Transillumination Score	Number of Matching Images in Initial Cohort
8	> 225	255	1
7	> 196	225	1
6	> 165	196	4
5	> 135	165	6
4	> 105	135	12
3	> 75	105	9
2	> 45	75	13
1	> 15	45	11
0	1	15	7

Table 2

Pairwise Comparisons of Reviewer versus Quantitative Image Rankings

Reviewer	Kendall's <i>tau</i>
Non-expert 1	0.83 ^{**}
Non-expert 2	0.87 ^{**}
Non-expert 3	0.85 ^{**}
Non-expert 4	0.81 ^{**}
Non-expert 5	0.82 ^{**}
Non-expert 6	0.88 ^{**}
Non-expert 7	0.85 ^{**}
Expert 1	0.72 ^{**}
Expert 2	0.77 ^{**}
Expert 3	0.85 ^{**}

^{**} Result significant to p-value $< 2.2 \times 10^{-16}$