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Treatment Responses to Tooth Whitening in Twins

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The aim of this study was to determine heritability estimates of treatment responses to a 10% hydrogen peroxide strip-based whitening system in twins. Eighty-five twin pairs were randomly assigned to 10% hydrogen peroxide whitening strips or placebo strips without peroxide. Both twins (monozygotic or dizygotic) received the same treatment. Maxillary teeth were treated for 30 minutes twice daily for 7 days. Efficacy was measured objectively as L^* (light–dark), a^* (red–green), and b^* (yellow–blue) color change from digital images at baseline (Δ) and day 8. Heritability estimates for tooth whitening treatment responses for changes from day 8 to baseline were obtained using variance-component methodologies. Whitening treatment responses were highly heritable ($h^2 = 71.0$) for Δb^* and Δa^*($p < .0001$), but not for ΔL^* ($h^2 = 27.0$), which was essentially modulated by environmental factors. This study has demonstrated that both genetic and environmental factors significantly contributed to seven-day whitening treatment responses achieved with 10% hydrogen peroxide strips.

■ **Keywords:** twins, treatment responses, tooth whitening, heritability

Teeth aesthetics is of great importance to dental patients, which includes tooth color. Recent studies have suggested the importance of tooth color to patients where their degree of dissatisfaction with tooth appearance ranged from 28% to 34% of the patients surveyed (Odioso et al., 2000; Qualtrough & Burke, 1994). Factors influencing tooth color may include genetic, congenital, metabolic, chemical, infectious, and environmental (Joyner, 2004). The color of the teeth is influenced by a combination of intrinsic and extrinsic colors due to stains that form on the tooth surface (Joyner, 2004; Watts & Addy, 2001). Inherited diseases may influence the thickness of enamel or the mineral and organic content of the enamel and therefore can affect tooth color (Simmer & Hu, 2001).

Most individuals using home bleaching for tooth whitening experience some lightening of their teeth even though it may not be to the extent they desire. In vitro studies have shown that teeth that had been extracted within 3 months and displayed comparable color at baseline exhibited wide variation in treatment responses to tooth whitening (Luk et al., 2004). Accordingly, anecdotal reports by practitioners suggest that not all patients are responsive to tooth whitening treatments, and that not all patients respond at the same rate.

The relevance of the twin study model to dissect the relative contribution of genetic and environmental components in assessing treatment responses to a particular intervention has been rarely utilized in medicine (Hohler et al., 2002), and to date has not been employed in dentistry. The aim of this study was to determine variation to tooth whitening treatment responses in twins by dissecting the relative contribution of genetic and environmental factors for parameters of tooth color.

Methods

Participants/Entry Criteria

A total of 85 pairs of twins were enrolled into the study. Among the twins, there were 42 monozygotic (MZ) pairs (23 female and 19 male pairs) and 43 dizygotic (DZ) pairs (13 male–male, 9 female–female and 21 male–female pairs). The age of the MZ pairs ranged from 12 to 26 years and that of the DZ pairs ranged from 12 to 29 years. Aspects of the research pertaining to the study protocol and informed consent were reviewed and approved by the New York University Institutional Review Board.

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The target population consisted of twins (1) in good general health; (2) with no vital bleaching history and no current tooth sensitivity; (3) who possessed a minimum of five maxillary anterior teeth without restorations; (4) with absence of fixed orthodontic appliances on the maxillary arch; (5) who did not present with meaningful malocclusion that would impact treatment or wearing of study whitening strips; and (6) with no severe periodontal disease, or who were not undergoing current treatment for periodontitis or within the past 6 months. None of the participants were current smokers and no dietary assessments were performed in this cohort.

Treatment Groups

This study was a part of a randomized double-masked placebo-based clinical trial on the efficacy of a 10% hydrogen peroxide gel on a flexible whitening strip (Crest® Whitestrips Premium, The Procter & Gamble Company, Cincinnati, OH) on tooth whitening parameters. Each twin pair was randomly allocated to receive either a 10% hydrogen peroxide whitening strip or placebo strips (0% hydrogen peroxide gel on a flexible whitening strip) for 1 week of use. There were 21 MZ pairs assigned to each group, while 21 and 22 DZ pairs used test strips and placebo strips respectively.

Zygosity Assessment

DNA was extracted from peripheral venous blood samples. DNA marker loci were PCR amplified using standard methods. Amplification products were detected by blood kits (QIAamp, Qiagen Inc., Valencia, CA, USA) with an ABI-377 fluorescent sequencer and analyzed by GENESCAN 2.1 (Applied Biosystems, Foster City, CA, USA; Zhang et al., 2001). Zygosity was determined by genotyping all individuals for eight highly polymorphic DNA loci (on chromosomes 2, 7, 11, 17, and 20). Individuals discordant for one or more markers were considered DZ.

Study Protocol

Participants underwent training sessions with multimedia visual aids prior to study commencement. Strip application was once a day supervised for 30 minutes (participants came to a dedicated research center for product application) and once a day unsupervised for 30 minutes, over a seven-day period. Participants were provided with seven maxillary strips, sufficient for 7 days at-home unsupervised use. Each strip was packaged in an individual white foil pouch, with the participant's identification number and investigator's contact information. Test products were supplied in individual kit boxes, with each kit containing either a seven-day supply of a 10% hydrogen peroxide flexible whitening strip or a seven-day supply of a placebo strip, along with a toothbrush and toothpaste. In addition, a digital timer was included in individual kits. Participants were asked to bring used strips that were generated during unsupervised procedures so as to assess compliance.

Tooth Color/Shade Digital Imaging

Twins were evaluated at baseline, and again at day 8 to ascertain clinical responses to tooth whitening (Δ = day 8 — baseline changes) by objective assessment of color parameters with standard digital images. After fixing positioning using a chin rest, images were obtained under standard polarized lighting conditions with a high resolution digital camera (Fuji HC1000 CCD, Fuji Photo Film Co., Tokyo, Japan) and motorized zoom lens linked to a personal computer. Red–green–blue values were determined relative to a calibration standard for each tooth pixel on the facial surfaces of the six maxillary anterior teeth. These values were averaged and transformed to an international standard for a three-dimensional color space, as b^* (yellow–blue), L^* (light–dark), and a^* (red–green). This quantitative method has previously been demonstrated to have measurement sensitivity and perceptual relevance in clinical studies involving different peroxide concentrations and delivery systems (Ferrari et al., 2004; Gerlach et al., 2000, 2002).

Statistical Analysis

Between-group statistical comparisons of color change (ΔL^*, Δb^*, and Δa^*) used analysis of covariance (ANCOVA), with baseline color and age as covariates. A term was included in ANCOVA for treatment by zygosity interaction. In all cases this was not statistically significant, and indicates that the mean response to treatment was not different due to zygosity. Supplemental analyses looked at the treatment differences for DZ and MZ pairs separately and confirmed that the treatment differences were nearly identical for the twin types. Variance component analysis adjusted by age and gender further examined baseline and changes from baseline for tooth color parameters by treatment groups, families within treatments, and within twin sets.

Heritability estimates ($h^2 = V_G/V_P$, where V_G is additive genetic variance and V_P is total phenotypic variance) for changes in color parameters (ΔL^*, Δb^*, and Δa^*) were obtained using the variance–component methodology implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package, version 8.0, available at: http://www.sfbr.org.solar/. This method utilizes maximum likelihood estimation procedures to provide estimates of V_G and V_P assuming a bivariate normal distribution of phenotypes in twin pairs. The method also estimates the mean levels of relevant variables accommodating a regression model that assumes a particular set of explanatory covariates (Almasy & Blangero, 1998). The null hypothesis (H_0:$V_G = 0$) of no heritability was tested by comparing the full model, which assumes genetic variation ($V_G \geq 0$), and a reduced model, which assumes

TABLE 1
Variance Components by Zygosity for Each Color Parameter

		DZ twin pairs ($n = 43$)		MZ twin pairs ($n = 42$)	
Color	Variance component	BL	Change from BL	BL	Change from BL
b*	Treatment groups	0.00	5.59	0.00	5.03
	Families within treatment	0.97	0.16	1.83	0.39
	Within twin sets	1.40	0.80	0.52	0.19
L*	Treatment groups	0.00	2.81	0.04	3.64
	Families within treatment	0.57	0.06	1.07	0.20
	Within twin sets	1.62	0.54	0.71	0.48
a*	Treatment groups	0.00	0.61	0.00	0.81
	Families within treatment	0.10	0.02	0.29	0.11
	Within twin sets	0.29	0.17	0.07	0.05

Note: Models are adjusted for age and gender; DZ = dizygotic; MZ = monozygotic; BL = baseline.

TABLE 2
Heritability Estimates for Treatment Responses to Tooth Whitening

Heritability (h^2)	ΔL*	Δa*	Δb*
	0.27	0.71	0.71
SE	0.12	0.07	0.08
p-value	.018	<.001	<.001
Sex	Covariates *p*-value		
	NS	NS	NS
Age	NS	NS	<0.001
Treatment	<0.001	NS	<0.001
% of variance due to covariates	0.69	0.62	0.76

no genetic variation (i.e., $V_G = 0$), via likelihood ratio tests. Heritability estimates were adjusted for age, gender, and treatment groups.

Results

Variance components that included treatment assignments, families, and twin sets were examined individually so as to split the variability in ΔL*, Δb*, and Δa* color parameters. For b-color parameter (primary whitening outcome measure) the components can be seen in Table 1. Of interest is the variance (twin sets) for change in color from baseline for Δb* for DZ twin pairs (0.80) and for MZ twin pairs (0.19). This means that although both DZ and MZ groups did not differ in treatment responses to whitening, the variance of these responses was less pronounced in MZ twins than in DZ twins, suggesting a genetic effect on treatment responses to tooth whitening procedures for parameter Δb*. These same patterns were found for Δa*, whereas ΔL* was about the same for DZ and MZ pairs (Table 1).

Analysis of heritability estimates confirmed these initial analyses (Table 2). Whitening treatment responses were highly heritable ($h^2 = 71.0$) for Δb* and Δa* ($p < .0001$), but not for ΔL* ($h^2 = 27.0$), suggesting a significant ($p = .018$) environmental component.

Discussion

This is the first study in the dental literature to assess the relative contribution of genetic and environmental factors to variation in treatment responses to a particular therapy (whitening) using the twin study model. It is important to note that variance component analyses and heritability estimates provided by this study are inherent to the population being studied and at present cannot be readily extrapolated to the general population.

Our results have unequivocally shown that changes in tooth color parameters are under genetic influence. The a* value is a measure of redness or greenness, and the b* value is a measure of yellowness or blueness. The high heritability estimates and decreased variance for MZ twin pairs for these parameters after whitening procedures was evident (Tables 1 and 2). In contrast, the L* value that measures the lightness of an object was modulated by environmental factors. There appears to be a paucity of data in the literature regarding the nature and chemical composition of colored materials found within dental hard tissues and the mechanistic effects on these structures. There are other factors that include type of bleach, concentration, time, heat, light, and age that can potentially influence whitening procedures and were not taken into consideration in our current study.

With the exception of syndromic disorders that affect tooth color such as *amelogenesis* and *dentinogenesis imperfecta*, there is no description in the literature of genes or regulatory mechanisms that may affect tooth color and lightness in humans. Animal studies suggest that genetic factors may contribute to tooth appearance after exposure to fluoride and, that Nrf2-deficient mice show alterations on color on the incisors due to iron content (Everett, 2011; Yanagawa et al., 2004).

Even though many of the proteins and proteinases secreted into the developing enamel matrix have been identified, the signaling pathways, growth factors, and receptors that control the developmental process and ameloblast differentiation are not known (Everett, 2011; Simmer et al., 2010). These processes are likely to be involved in the

genetic determination of enamel thickness and conceivably may influence tooth color.

The results of this study afford the possibility to consider issues related to optimal dosage for treatment efficacy for any therapeutic dental product by employing the twin study model. This may be of value in the future to aid clinicians in the process of their clinical decision-making.

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