GENETIC AND ENVIRONMENTAL FACTORS IN AGE-RELATED NUCLEAR CATARACTS IN MONOZYGOTIC AND DIZYGOTIC TWINS

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ABSTRACT

Background Age-related cataracts are a major public health problem. The relative importance of genes and environment in the causation of nuclear cataracts, the most common form of age-related cataracts, is not known.

Methods We studied 506 pairs of female twins (226 monozygotic and 280 dizygotic) who were 50 to 79 years old (mean, 62). The amount of nuclear cataract in the right and left eyes was determined objectively by analysis of Scheimpflug lens photographs (yielding three measures) and subjectively with use of the Oxford Clinical Cataract Classification and Grading System (yielding one measure). All eight measures (four in each eye) were subsequently combined in one summary measure of nuclear cataract for each woman. A univariate maximum-likelihood model was used to estimate the variance of the genetic and environmental contributions to each of the measures.

Results The different measures of cataract formation were highly correlated (correlation coefficients, 0.71 to 0.94). The mean scores were similar for the right and left eyes and for monozygotic and dizygotic twins. Quantitative genetic modeling of each of the nuclear-cataract scores invariably resulted in a best-fitting model that involved additive genetic effects, unique environmental effects, and age. The common environmental and dominant genetic effects could be removed from the models without significant loss of fit. The overall heritability in the combined nuclear-cataract score (the proportion of the variance explained by genetic factors) was 48 percent (95 percent confidence interval, 42 to 54 percent); age accounted for 38 percent of the variance (95 percent confidence interval, 31 to 44 percent) and unique environmental effects for 14 percent (95 percent confidence interval, 12 to 18 percent).

Conclusions Genetic effects are important even in such a clearly age-related disease as nuclear cataract, explaining almost 50 percent of the variation in the severity of this disease. (N Engl J Med 2000;342:1786-90.)

CATARACT is a major public health problem. The World Health Organization estimates that 45 million people in the world are blind, about half of them as a result of cataracts.[1] The only treatment is extraction of the cataract, which is performed 1.5 million times each year in the United States.[2] Cataract, defined as opacity within the clear lens of the eye, occurs mainly in the nuclear, cortical, and posterior subcapsular regions of the lens. Nuclear cataracts are the most common type of age-related cataract leading to surgery.

Research into nuclear cataracts has focused mainly on environmental factors. Age, female sex, and smoking seem to be the most important risk factors. Other factors, such as diet, exposure to sunlight, estrogen sufficiency or deficiency, and cardiovascular factors, have been associated with the frequency of nuclear cataracts in some but not all studies.[3,4] The possibility that genetic influences may be involved in the development of age-related nuclear cataracts has been largely ignored, although mutations associated with congenital cataracts in humans[5] and nuclear cataracts in mice[6,7] suggest that genes have a role.

The study of twins has been described as the "perfect natural experiment" in which to determine the relative importance of genetic and environmental factors.[8] To estimate the heritability of a feature, the concordance or correlation of the feature between identical (monozygotic) twins and nonidentical (dizygotic) twins is measured, and the magnitude of the concordance or correlation in the two types of twins is compared. We determined the amount of nuclear cataract in a large sample of adult female twins in order to estimate the relative role of genes and environment in the causation of such cataracts.

METHODS

Subjects

We studied 506 pairs of white female twins (226 monozygotic and 280 dizygotic) who were 50 to 79 years of age. The women were initially recruited to participate in the St. Thomas' United Kingdom Adult Twin Registry and were unaware of any hypotheses or proposals for specific studies[9]; only later were they invited to have an eye examination. Zygosity was determined by a standardized questionnaire[10] and confirmed by DNA analysis of short tandem-repeat polymorphisms in the 196 pairs for which there was any doubt about zygosity. Smoking status was assessed. The 1012 women had a total of 2024 eyes; data from 49 eyes were excluded from full analysis. Of these 49 eyes, 24 had pseudophakia from previous cataract surgery, 11 could not be evaluated because of previous eye surgery or injury, and Scheimpflug images for objective grading of 14 eyes were missing, although they were subjectively scored with the Oxford Clinical Cataract Classification and Grading System.[11] For each ungraded eye, the corresponding eye of the twin was excluded from the analysis, because the analysis involved comparison within pairs of twins. Our institutional ethics committee approved the study, and all the women gave informed consent.

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Measurements

Lens grading was performed approximately one hour after dilation of both pupils with 1 percent tropicamide and 10 percent phenylephrine. A single investigator, who performed all grading, saw each pair of twins together and was therefore aware of their probable zygosity. Each eye was graded according to both a subjective and an objective grading system to generate scores of nuclear opacity; since opacity is a continuous measure, it would be artificial to divide the subjects into a group with and a group without cataract for analysis. The degree of opacification is an indication of the amount of cataract; the measures are referred to as nuclear-cataract scores in this population study, even for women who did not have clinically important cataracts.

The subjective grading was based on slit-lamp measurements of "white scatter," according to the Oxford Clinical Cataract Classification and Grading System.[11] White scatter is a measure of the extent to which light is scattered back when shone into the lens and is an indication of the amount of nuclear cataract. Brunescence (brown nuclear discoloration seen in lenses with cataract) was not included, because it is the most difficult of the subjective measures to grade accurately, and it is distinct from nuclear cataract causing visual impairment.[12,13] White scatter was estimated by viewing the lens in a standardized fashion (slit-lamp illumination on full power at 45 degrees with a slit width of 0.3 mm, with the same slit lamp used for all women) and comparing it with five reference standards based on neutral-density gray-scale samples. Each lens was given a score from 0 (indicating a clear lens with no opacity) through 5 (the most opaque, light-scattering lens); gradations of 0.1 were used to improve the detection of differences.[14] This grading system is reproducible,[15] and white-scatter scores are comparable to scores for "nuclear opalescence" in other subjective grading systems, such as the Lens Opacities Classification System.[12,16]

In addition to the subjective system, an objective grading system was used because of the difficulty of subjectively grading early lens opacities[13] and the potential bias due to the examiner's knowledge of the twins' zygosity when they were seen at the same time. A Scheimpflug camera system developed in Oxford was used (Marcher Diagnostics, Hereford, United Kingdom).[17] The system is based on a slit-lamp camera modified to obtain photographs with the entire anterior segment in focus.[18] Digitized images are obtained in a dark room with standardized gain and exposure and stored in a computer. Analysis of these images allows the pixel density of selected areas of the lens to be measured, resulting in reproducible nuclear-cataract scores,[19] which have been used in other cataract studies.[17,20,21] An example of a Scheimpflug image of the lens of one woman is shown in Figure 1, with the densitometric measures (central nuclear dip, anterior peak, and nuclear average) superimposed. Central nuclear dip is the density in the center of the lens nucleus; anterior peak is the greatest density in the interior half of the nucleus; and nuclear average is the average density across the whole lens nucleus.

A study of intraobserver reproducibility performed in 15 pairs of twins at least one month after the initial assessment found that the scores based on Scheimpflug images were reproducible. The intraclass correlations for right-eye measures were 0.97 for central nuclear dip, 0.90 for anterior peak, and 0.96 for nuclear average; the left-eye scores were similar. The intraclass correlations for scores on the Oxford Clinical Cataract Classification and Grading System were good for white scatter (0.80 in the right eye and 0.76 in the left eye).

The individual measures of nuclear cataract showed a left-skewed distribution and were therefore log-transformed before analysis to produce approximate normal distributions.

Statistical Analysis

Factor Analysis

Factor analysis was used to test the extent to which the various measures of nuclear-cataract scatter from the Oxford system and the Scheimpflug scores for both the right and the left eyes measured the same phenotype and could therefore be combined into one measure for each woman.[22,23] This combined nuclear-cataract score was then used as a continuous variable in univariate model-fitting analysis of heritability to obtain a single estimate of heritability for nuclear cataracts.[24]

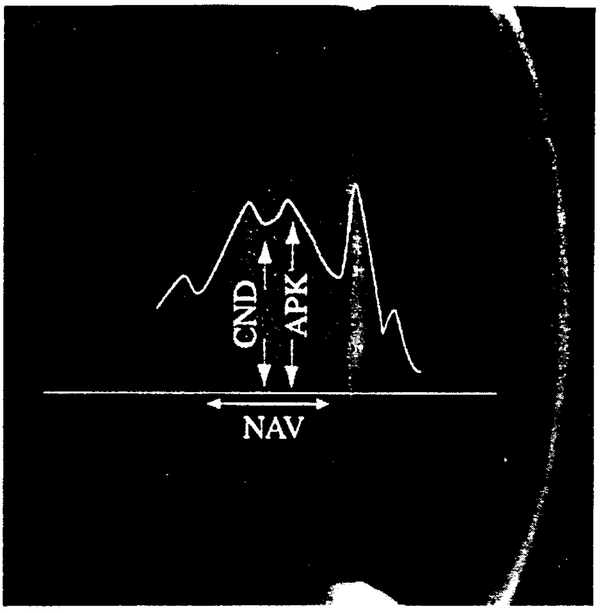

Figure 1. Scheimpflug Image of the Lens.

The superimposed white curve shows the pixel density of a rectangular region of interest that is 20 pixels in height along the axial center of the nucleus. Three scores were derived from these images: the central nuclear dip (CND) and anterior peak (APK) from one image, and the nuclear average (NAV) from a second image taken at a different standardized gain. The highest peak of the curve shows the scatter from the cortex of the lens.

Model-Fitting Procedure

The details of fitting models to data on twins have been described elsewhere.[25,26] In short, an individual person's phenotype is the sum of the effects of both genotype and environment. To study the sources of individual differences (i.e., the variance) in a phenotype, genetically related subjects are required, and twins are such a group. Monozygotic twins have the same genes, and dizygotic twins share on average 50 percent of their segregating genes. It is assumed that both twins have roughly the same family environment,[27] and that therefore any greater similarity between monozygotic than dizygotic twins is due to genetic influences.

Quantitative genetic model fitting, now standard in studies of twins, is based on comparison of the covariance (or correlation) in the measurement of the trait in question between monozygotic and dizygotic twins. It allows separation of the observed phenotypic variance into additive and dominant genetic components and into common and unique environmental components. The common environmental component estimates the effect of the shared family environment, whereas the unique component applies only to the individual person and includes measurement error. Dividing each component by the total variance yields the standardized component of variance — for example, the heritability, which can be defined as the ratio of additive genetic variance to total phenotypic variance.

Since age is by definition a risk factor for age-related nuclear cataracts and twins have the same age, the correlation between twins in the frequency of age-related cataract will be inflated for both monozygotic and dizygotic twins. If not accounted for, the effect of age will be confounded with that of their common environment.[28] To eliminate this problem, and to permit the estimation of its effect on the variance within the population, we incorporated age into the model. The twin model used for analysis is shown in Figure 2.

The significance of additive genetic factors, the common environment, dominant genetic factors, and age as components of the variance was assessed by removing each sequentially and testing the deterioration in the fit of the model. This procedure leads to submodels that explain the variance and covariance with as few variables as possible. The submodels were compared with the full model by hierarchic chi-square tests. The difference in values between the submodel and the full model is itself approximately distributed as chi-square, with the degrees of freedom equal to the difference in degrees of freedom between the submodel and the full model. Data handling and preliminary analyses were carried out with Stata software.[29] All genetic modeling was carried out with Mx software.[30]

RESULTS

The mean (±SD) age of the 226 monozygotic pairs of twins was 62±6 years (range, 51 to 75 years) and that of the 280 dizygotic pairs of twins was 62±6 years (range, 49 to 79). The scores for each of the measures of nuclear cataract and the number of pairs of twins included in each analysis are shown in Table 1. The scores were similar for the right and the left eyes and for monozygotic and dizygotic pairs.

The different measures of cataract were highly correlated. The correlation coefficients for the three measures taken from the Scheimpflug images (central nuclear dip, nuclear average, and anterior peak) ranged from 0.87 to 0.94. The scores for white scatter, the subjective estimate of the same phenomenon, were correlated with the scores from the image analysis (correlation coefficients, 0.71 to 0.78). The correlation between right and left eyes ranged from 0.86 to 0.93 for all scores.

Factor analysis identified only one factor that explained over 92 percent of the variance for all eight measures of nuclear scatter (the three Scheimpflug scores and white scatter for each eye). This finding suggested that the different cataract measures were a reflection of the same phenotype. A single combined score for nuclear cataract was therefore calculated for each woman.

The scatter plots of the combined scores for nuclear cataracts for one twin against the combined scores of the other are shown in Figure 3. The correlation within pairs was higher for monozygotic than for dizygotic twins. The correlation coefficients were 0.90 and 0.57, respectively, which were similar to the correlation coefficients for each of the eight separate measurements (range, 0.77 to 0.88 for monozygotic twins and 0.44 to 0.59 for dizygotic twins). The higher correlation for monozygotic than for dizygotic twins suggests that genes have an important causative role. The correlation for dizygotic twins, which is also relatively high, may represent the importance of age or shared environment.

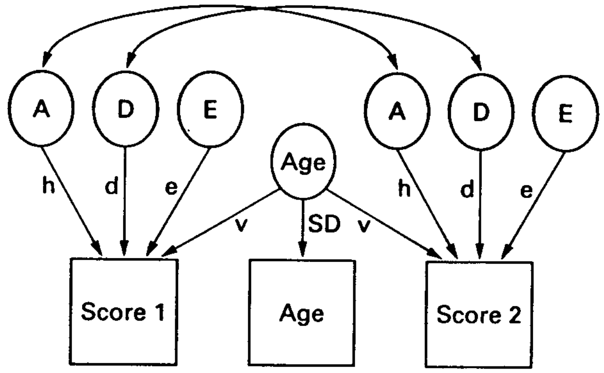

Figure 2. Path Model for the Nuclear-Cataract Scores for Twin 1 and Twin 2 (Score 1 and Score 2) and Age.

Observed scores are represented in squares. Latent factors, which the study attempts to estimate, are represented in circles. A, D, and E are the additive genetic, dominant genetic, and unique environmental influences, respectively. C, the common environmental influence, was also tested but is omitted in order to simplify the diagram. Monozygotic twins share the same genes, and dizygotic twins share half their additive genes. Therefore, the correlation between the latent additive genetic factors is 1.0 for monozygotic twins and 0.5 for dizygotic twins. Similarly, because dizygotic twins share only one fourth of the dominant genetic effect shared by monozygotic twins, the correlations are 1.0 and 0.25 for dominance for the monozygotic and dizygotic twins, respectively. The regression coefficients of the observed variables on the different latent factors are shown in lowercase letters: h denotes the additive genetic effect, v the age effect, d the dominant genetic effect, and e the unique environmental effect. SD denotes the standard deviation of age.

Univariate model-fitting analysis with the single combined nuclear-cataract score for each woman revealed that the effect of common environment could be removed from the model with no significant loss of fit. The contribution of the dominant genetic component could also be removed from the model without significant loss of fit. However, the effects of age and of the additive genetic component could not be eliminated ($P<0.001$ for both comparisons). This finding suggests that the best-fitting model explains variance in the population by the effects of additive genetic factors, unique environmental factors, and age. This was also the best model for each of the eight individual measures (data not shown).

Estimates based on this model resulted in a value for heritability in the combined nuclear-cataract score of 48 percent (95 percent confidence interval, 42 to 54 percent); the remaining variance was explained by age (38 percent; 95 percent confidence interval, 31 to 44 percent) and unique environmental effects (14 percent; 95 percent confidence interval, 12 to 18 percent). This result was similar to that obtained by univariate model-fitting analysis for each of the measures of nuclear cataract; heritability accounted for 44 to 47 percent of the variance in the scores based on

TABLE 1. MEAN (±SD) NUCLEAR-CATARACT SCORES FOR THE RIGHT AND LEFT EYES OF MONOZYGOTIC AND DIZYGOTIC TWINS.

MEASURE	EYE	MONOZYGOTIC PAIRS		DIZYGOTIC PAIRS	
		SCORE	NO.*	SCORE	NO.*
Central nuclear dip†	Right	60±16	212	58±14	272
	Left	59±16	215	57±14	265
Anterior peak†	Right	69±16	213	67±15	272
	Left	66±17	215	64±15	265
Nuclear average†	Right	68±13	213	67±12	272
	Left	67±13	215	65±11	265
White scatter‡	Right	2.2±0.4	217	2.1±0.4	274
	Left	2.2±0.4	221	2.1±0.4	269

*The number of pairs of twins included in the analysis after exclusions is given. Numbers of twins for some measures are low because of missing images.

†The score is the pixel density of the relevant area of the lens image (see Fig. 1). Scores ranged from 20 (almost clear lens) to 160 (densely cataractous lens).

‡The white-scatter scores, based on subjective grading, range from 0 (indicating no scatter) to 5 (densely cataractous lens).

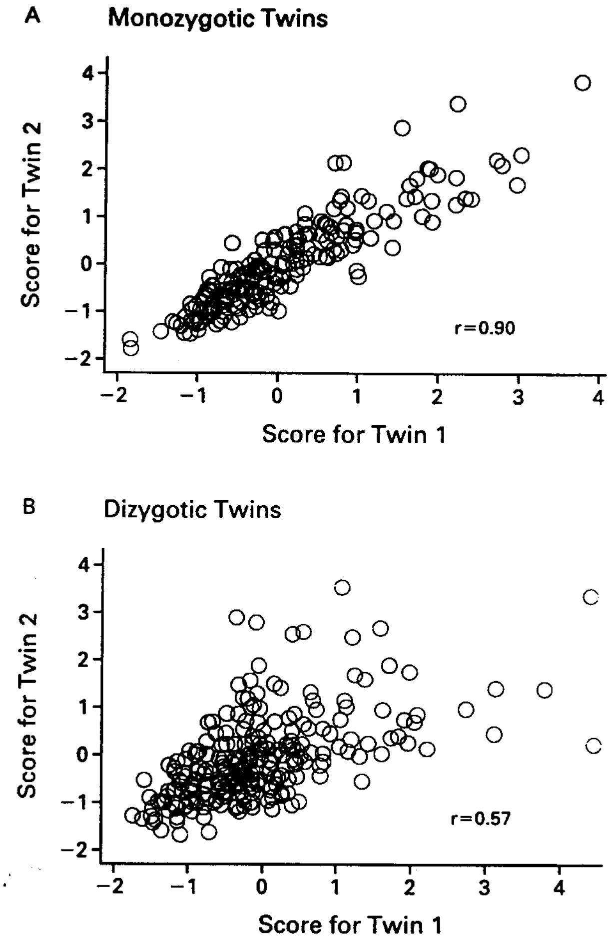

Figure 3. Scatter Plots of the Combined Nuclear-Cataract Scores. The scores for one twin are plotted against the scores for the other twin in monozygotic pairs and in dizygotic pairs.

Scheimpflug images and accounted for 49 and 52 percent of that in the white-scatter scores for the left and right eye, respectively.

DISCUSSION

This study of nuclear cataracts in twins demonstrates that genes are important in this age-related condition, with a heritability of 48 percent for nuclear scatter. Age accounted for 38 percent of the variance among these women, and the unique environment, which includes factors such as smoking, for only 14 percent. This low estimate of the proportion of cataract that is attributable to unique environmental factors may explain some of the difficulties in identifying environmental risk factors for cataracts and in obtaining significant outcomes in intervention trials. A segregation analysis has suggested that a single major gene could account for up to 35 percent of the variability in the frequency of nuclear cataract,[31] a result that supports a possible role for genes in causation.

The scores for the Scheimpflug images correlated well with each other and generated similar estimates of heritability (44 to 47 percent). The heritability of the white-scatter scores from the Oxford subjective grading system was similar (49 and 52 percent in the left and right eye, respectively). All these scores measure the same phenomenon — the scattering back of light transmitted into the eye — which is a measure of the amount of opacification within the lens nucleus. We therefore think that combining these measures by factor analysis to obtain a single value for the heritability of nuclear cataracts is justified. Factor analysis allows for the obvious interdependence of the measures, both within and between eyes; because these measures were strongly but not perfectly correlated, all four measures in each eye of each woman contributed to the combined score.

Classic twin studies have limited power to detect dominance.[32] Although it did not contribute significantly to the model, the estimated contribution of dominance in the model incorporating additive and dominant genes and unique environmental effects was 19 percent of the variance. The genetic contribution to the variance might therefore include some effect of dominant genes, which our model might not have been powerful enough to detect.

Although the results in the whole sample of twins were analyzed, the twins in this study were volunteers. Thus, there was a potential for recruitment bias, which was minimized by recruiting the twins before the eye test was proposed. We studied only women, because cataracts are more common in women and also because including only women provided a more homogeneous group. Data on eyes that had previously undergone cataract surgery were excluded from the analysis (24 eyes), because no continuous data could be derived from them.

There is no evidence that twins have more or fewer cataracts than singletons, and in general, morbidity and mortality are similar in twins and in singletons.[27] We therefore believe that the conclusions from this study of twins can be generalized to the whole population. However, estimates of heritability are population-specific; our figure applies to a population of middle-aged-to-elderly British white women and may differ for other ethnic or racial groups or those with different environmental circumstances.

This study of the heritability of cataracts did not address individual confounding variables or environmental effects. For nuclear cataracts, the most important environmental effect is smoking. Simulations have shown that for a disease with familial aggregation, familial clustering of environmental risk factors that impose a relative risk up to 10 are unlikely to influence heritability significantly.[33] Reanalysis of our data, after elimination of the effects of smoking, altered heritability by only 1 percent. Gene–environment interactions, which were assumed not to be present in the twin model, would not significantly alter the heritability of the disease in this population, if it were present, although such interaction might make possible the prevention of a disease in an individual person, even if that disease were strongly genetic.

Understanding of how genetic mechanisms can result in age-related nuclear cataracts may be furthered by information about the specific genetic defects that are now being isolated in patients with congenital cataracts[5] and in specific adult-onset cataract syndromes, such as that associated with myotonic dystrophy.[34] Our results should encourage others to search for genes in patients with age-related nuclear cataracts through linkage and candidate-gene studies.

In conclusion, the heritability of age-related nuclear cataracts is substantial, whereas a person's unique environment accounts for little of the variance. Most of the remaining variance is explained by age.

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