

Repeated Measures of Intraocular Pressure Result in Higher Heritability and Greater Power in Genetic Linkage Studies

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PURPOSE. To analyze the effect of using one reading, the mean of two readings (from the same eye), or the mean of four readings (two from each eye) on the heritability estimates of intraocular pressure (IOP). This was a cohort study in which 344 pairs of twins, 163 monozygotic (MZ) and 181 dizygotic (DZ), were enrolled.

METHODS. IOP was measured using three tonometers: the gold standard Goldmann applanation tonometer (GAT), the Ocular Response Analyzer (ORA; Reichert Buffalo, NY), and the Dynamic Contour Tonometer (DCT, Pascal; Swiss Microtechnology AG, Port, Switzerland). The main outcome measure was the heritability of IOP correlated with the number of measurements.

RESULTS. The mean IOPs of all four readings with the three tonometers were: 14.1 ± 2.9 mm Hg for GAT, 15.9 ± 3.2 mm Hg for ORA, and 16.9 ± 2.7 mm Hg for DCT. As the number of readings increased, the calculated heritability (b^2) of IOP measured using the GAT readings increased from 0.56 for one reading (95% confidence interval [CI], 0.44–0.65) to 0.58 for the mean of two readings (95% CI, 0.46–0.67) to 0.64 for the mean of all four readings (two right and two left; 95% CI, 0.55–0.72). Similar results were seen with the other two instruments.

CONCLUSIONS. The results demonstrated that the use of the mean of several readings from both eyes reduced measurement error, yielding a higher heritability estimate. The higher heritability would increase the power to detect linkage in a genome-wide analysis. (*Invest Ophthalmol Vis Sci.* 2009;50:5115–5119) DOI:10.1167/iovs.09-3577

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There is increasing interest in the genetic component of common, complex age-related diseases.¹ Currently identified glaucoma-susceptibility genes contribute to pathogenesis in only a minority of cases.^{2–4} Given the relative rarity of glaucoma in the population, researchers have focused on the intermediate traits of glaucoma such as intraocular pressure (IOP). The heritability of IOP, defined as the proportion of variance attributed to genetic factors, has been estimated in family and twin studies to range between 0.30 and 0.64. In all these studies, the IOP from only one eye was used.^{5–10} In one of them, the higher IOP measurement from either eye was used for quantitative analyses.⁶ Of the published linkage analyses for IOP, data from one eye was used in two studies,^{11,12} whereas in another study, measurements were taken in duplicate on each eye, and the average was used.¹³

Ophthalmic research poses a question as to whether to use one eye or the data collected from both eyes. In certain disorders, such as choroidal melanoma where only one eye is affected in 99% of cases,¹⁴ it is not possible to use the data from both eyes. Where there are measures from both eyes such as IOP, the use of only one eye for statistical analysis is valid, but inefficient.¹⁵ Bias may be introduced if data from one eye are actively selected (such as highest IOP) from data available from both eyes. The use of data from one eye per individual avoids the statistical analysis complication of high correlation between eyes, but there may be a considerable waste of available data.¹⁵ Newcombe and Duff¹⁶ concluded that averaging the data from both eyes gives greater precision and greater power to detect a difference of a given size.

Heritability allows a comparison of the relative importance of genes and environment on the variation of traits within and across populations. It can be defined as a ratio of variances—specifically, as the proportion of total variance in a population for a particular measurement, taken at a particular time or age, that is attributable to variation in additive genetic or total genetic values—termed the narrow-sense heritability (or just heritability, b^2) and the broad-sense heritability (H^2), respectively.¹⁷ Repeated measurements of an individual can be taken for some traits such as IOP. If it is assumed that these repeated measures are expressions of the same genotype, then the variation within individuals is caused by measurement error and other random environmental factors.¹⁷

Twin studies have been widely used in heritability studies. We set out to analyze the effect of using one reading, the mean of two readings (from the same eye), or the mean of four readings (two from each eye) on the heritability estimates of IOP, using three different instruments in a large cohort of twins. The three tonometers used were the gold standard Goldmann applanation tonometer (GAT), the Ocular Response Analyzer (ORA; Reichert, Buffalo, NY) and the Dynamic Contour Tonometer (DCT; Pascal; Swiss Microtechnology AG, Port, Switzerland).

METHODS

Three hundred forty-six pairs of healthy twins, mean age of 57.5 years (range 16–84), were recruited from the TwinsUK Adult Twin Registry, based at St. Thomas' Hospital, London. The subjects were twin volunteers from the general population, and were part of a twin study on glaucoma heritability,¹⁸ although they were recruited for studies other than eye studies and subsequently asked to attend for an eye examination. Historically, the TwinsUK registry was established to investigate predominantly female disorders, such as osteoporosis. Since then, the number of males recruited has increased, but the majority of twins who volunteer are female, consistent with other volunteer twin registries. All subjects provided informed consent, in accordance with the Declaration of Helsinki, and the study was reviewed by the Local Research Ethics Committee. Six hundred ninety-two individuals had two readings performed on both eyes with all three instruments by a single investigator (FC). Twins of each pair were tested in immediate succession.

The ORA is a new type of noncontact air-puff tonometer that ejects 20 ms of air impulse and monitors the time course changes of the cornea by an electro-optical collimation detector system. The method of functioning of the ORA is described in more detail elsewhere.^{19,20} Goldmann correlated IOP (IOPg) readings were used in the study. The DCT is a contact tonometer with a concave surface with a radius of curvature of 10.5 mm, which creates a distribution of forces between the central contour of the tip and the cornea that equals the forces generated by the internal pressure of the eye.²¹ It gives a measure of IOP that is independent of CCT.

A drop of proxymethacaine 0.5% with fluorescein was instilled in both eyes before testing. IOP measurement with the ORA was performed first, as it is a noncontact device. First and second readings were taken in the right eye and, if the accuracy was poor, a third reading was taken, with a repeat of the process for left eye. Poor accuracy was determined by an abnormal graph display or an abnormally high or low IOP measurement. The second instrument used was the DCT. In this case, two readings per eye were again taken and the readings were alternated between the eyes. Only those measures with a reliability score recommended by the manufacturer (≤ 3 on a scale of 1 to 5) were accepted (mean reliability, 1.8 ± 0.9). The last readings were taken with the GAT. For these readings, the gauge was set at 0 mm Hg and dialed up to the twin's IOP and was repeated on the contralateral eye. The dial was next set at 40 mm Hg and moved down to the individual's IOP to provide a second reading. An approximate interval of 2 minutes was allowed between each instrument. The possibility that multiple measurements in succession caused lower IOP readings cannot be excluded, but it is felt that this gap between instrument measurements should have kept any possible lowering of IOP to a minimum.

Data handling and preliminary analyses were undertaken with commercial software (Stata; Intercooled Stata for Windows 95, ver. 5.0; StataCorp, College Station, TX). Pearson correlation coefficients were calculated for monozygotic (MZ) and dizygotic (DZ) twin pairs, with a greater correlation for MZ pairs suggesting a genetic component to the trait in question. The covariance matrices for twin pairs were input into the Mx genetic modeling program (Virginia Commonwealth University, Richmond VA), which performs variance component estimations by maximum-likelihood methods. The observed phenotypic variance can be divided into additive genetic (A), dominant genetic (D), common environmental (C), and unique environmental (E) components. The common environmental component estimates the contribution of family environment, whereas the unique environmental component estimates the effects that apply only to each individual. The broad-sense heritability, which estimates the extent to which variation in these parameters in a population can be explained by genetic variation, can be defined as the ratio of genetic variance (A+D) to total phenotypic variance (A+D+C+E). The best-fitting model is calculated by the use of the Akaike information criterion. The Akaike information criterion describes the model with best goodness of fit combined with

parsimony (fewest latent variables) and is calculated as two times the degree of freedom minus the model fit χ^2 . The submodel with the lowest Akaike information criterion is the best fitting. Heritability was calculated for first readings from the right eye, the mean of the first and second readings from the right eye, and the mean of all four readings (two right and two left). Results for right and left eyes demonstrated no significant differences, and so right eye measures were arbitrarily used for analysis, as in the previous heritability studies.

The theoretical power to detect linkage and association was determined using the Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc/>) provided in the public domain by the Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA) formulated by Purcell et al.,²² which provides automated power analysis for linkage and association tests. In power calculations, assumptions are made, such as the proportion of variance explained by the trait locus, gene action, and marker heterozygosity.²² Other parameters that must be specified for a nonparametric variance components linkage analysis are the quantitative trait locus (QTL) heritability (i.e., the proportion of variance explained by the trait locus), the mode of inheritance at the locus, and the marker heterozygosity. When making these calculations, we did not test for dominance, and therefore it cannot be specified in the model. The module used was the QTL linkage for sibships; the number of sibships considered was two. The power to detect linkage with $\alpha = 0.05$ was taken, with the sample size required for 80% power. In our calculations, we also assumed that the recombination fraction was 0. In reality, although possible, this is rarely the case; however, the assumption was the same for all calculations. It therefore would not alter the results in terms of comparison of numbers of readings.

RESULTS

Of the 346 twin pairs examined, all were Caucasian and 91.5% were female. Only one reading per eye was available for two individual subjects and these, together with those of their twins, were therefore not included in the analysis. Hence, data were available for 344 pairs of twins: 163 MZ pairs (mean age, 50.2 years; SD, 15.5 [16.1–81.0]) and 181 DZ pairs (mean age, 55.1 years; SD, 9.7 [19.2–77.0]). Four subjects had received a previous diagnosis of glaucoma (0.6% of the cohort), and a further three subjects were referred to local ophthalmology services (0.4%) for further investigation for glaucoma based on examination findings in this study. Exclusion of these subjects did not alter the mean data and twin-pair correlations, and they were therefore included in the analysis.

Mean IOPs of all four readings for the three tonometers were: 14.1 mm Hg for GAT (SD, 2.9; range, 7.5–24.2), 15.9 mm Hg for ORA (SD, 3.2; range, 8.2–25.8) and 16.9 mm Hg for DCT (SD, 2.7; range, 11.3–27.2). The age and sex distributions are shown in Table 1, together with the means and range of IOP measured by all three instruments; MZ and DZ twin groups were similar. The twin-twin GAT correlations were higher in MZ than in DZ twin pairs for one reading (MZ, 0.47; DZ, 0.28), the mean of two readings (MZ, 0.54; DZ, 0.31), and the mean of four readings (MZ, 0.60; DZ, 0.40). The similar difference in correlations between MZ and DZ twins with the ORA and DCT are shown in Table 2. Notably, both MZ and DZ correlations increased as the number of readings increased, from one to the mean of two readings from both eyes.

Genetic modeling suggested the best-fitting model for both parameters to be the AE model, meaning that additive genetic effects and individual environmental effects explained the variance. Individual environmental effects may include factors such as measurement error and environmental exposures unique to the individual (as opposed to shared with their twin). The calculated heritability (b^2) of IOP from the GAT readings increased with increasing the number of readings

TABLE 1. Demographics for MZ and DZ Twins and the Mean IOPs for All Three Tonometers

	Monozygotic	Dizygotic
Number of twin pairs	163	181
Mean age (range), y	50.2 (16.1-81.0)	55.1 (19.2-77.0)
Female twins, %	95.6	89.8
GAT-IOP (range), mm Hg	13.7 ± 2.7 (7.5-22)	14.3 ± 2.9 (8.3-24.3)
ORA-IOP (range), mm Hg	15.6 ± 3.1 (8.2-26.6)	16.1 ± 3.2 (8.7-25.8)
DCT-IOP (range), mm Hg	16.9 ± 2.6 (11.3-26.9)	17.0 ± 2.8 (11.3-27.2)

from 0.56 (95% confidence interval [CI], 0.44-0.65) for one reading, to 0.58 (95% CI, 0.46-0.67) for the mean of two readings, to 0.64 (95% CI, 0.55-0.72) for the mean of all four readings (two right and two left). The remaining proportion of variance was due to individual environmental effects of 0.44 (95% CI, 0.33-0.53), 0.42 (95% CI, 0.35-0.56), and 0.36 (95% CI, 0.28-0.45) for one, two, and four readings, respectively. The results of the calculated b^2 with the three instruments are tabulated for comparison in Table 3. There was a significant difference in standard deviation for GAT, when comparing one reading and the mean of four ($P = 0.01$). The differences comparing one and the mean of two readings ($P = 0.15$) and comparing the mean of two to the mean of four readings ($P = 0.11$) were not statistically significant.

The power to detect linkage was calculated using the Genetic Power Calculator and the results are shown in Table 4. The theoretical sample size required to detect linkage (using the assumption of a power of 80% and α value = 0.05) was reduced with increasing numbers of readings. The power to detect linkage increased from one reading, to the mean of two, and to the mean of four, with all three instruments. For instance, in the GAT readings, the sample size reduced from 7825 to 5538 when the number of readings was increased from one to the mean of four (two from each eye).

DISCUSSION

This study has demonstrated that by using the mean of several IOP readings, the heritability estimate increases, in turn increasing the power for linkage analysis. The higher correlations between MZ than DZ twins support a significant genetic influence on IOP, and this has indeed been shown in two recent twin studies examining the heritability of IOP.^{9,23}

The increase in heritability was similar with three different tonometers for IOP, with an increase in the point estimate of heritability of 8%, 14%, and 10% for the GAT, ORA, and DCT, respectively, from one reading to the mean of four readings. Although there is obviously overlap of the 95% CIs, the point estimate of heritability is an important parameter that determines statistical power in gene-mapping studies that use pedigree information. It is also of interest to note that the standard deviation of the mean of four readings was significantly lower than that of a single reading. The standard deviation reflects the variance, and so the reduced variance reflects a lower measurement error, in turn reflected in the higher heritability estimate. A high heritability implies a strong correlation between phe-

notype and genotype, so that loci with an effect on the trait can be more easily detected. However, by itself, heritability does not provide information about the genetic architecture of the traits—for example, how many loci contribute to genetic variation.¹⁷ Heritability ranges from 0 (all variation environmental) to 1 (all variation due to genetic factors). One contributor to the individual environmental component is measurement error.

Independent of the measurement technique used, the heritability of IOP in this twin study was ~60%. The heritability values as calculated using the mean of two readings from one eye were 0.58, 0.61, and 0.59, for the GAT, ORA, and DCT, respectively. These are similar to the Finnish Twin Study of Aging estimation of 0.64 (95% CI, 0.53-0.71), which used noncontact tonometry in a smaller number of twins of average age 10 years older than those in this study.⁹ In a previously published study by our group, the estimated heritability using the mean of four readings with GAT was 0.62,²³ whereas in this present study it was 0.64. The reason for this slight difference in heritability estimates probably reflects a smaller group of twins studied: data were available on 422 pairs of twins for our first study, whereas for this present study only 344 pairs, who had measurements from all three instruments, were analyzed. The main scope of this current work was not to determine the heritability of IOP, but to examine the effect of increasing the number of readings and therefore reducing the measurement error on the heritability estimate. Heritability is a population-specific factor, and our study applies to this population of largely Caucasian British females; the results could be different for other populations. However, the principles of repeated measures resulting in reduced measurement error and higher heritability are likely to apply to all populations.

Other heritability estimates of IOP from family and sibling-based studies have been considerably lower, ranging from 0.29 to 0.50.^{5-8,10} The lower heritability values may reflect the fact that those studies were not twin-based. Twin studies tend to show a higher heritability, in part due to the shared common environment that twins have, such as age, intrauterine environment, and family background. From this study, it was demonstrated that by increasing the number of readings used to calculate the heritability, the value was increased.

It is widely acknowledged that upwards of 95% of the variation of a trait is within a population and the remainder of the variation is between populations.²⁴ Trait variation within and between populations results from genetic differences between people as well as environmental effects. These within- and between-population differences reflect underlying genetic and environmental variation. If errors have occurred in the measurement of the trait, due to either instrument or human measurement errors, they can compromise many statistical methods and may falsify the partitioning into genetic and environmental components.²⁵ Macgregor et al.²⁶ examined the effect of measurement error on heritability using height as the variable and comparing the heritability determined from clinical measures of height with that determined from self-reported height. They found that there was a bias toward overestimating

TABLE 2. Correlation between Twins

No. of Readings	ORA		DCT	
	rMZ	rDZ	rMZ	rDZ
1	0.56	0.28	0.59	0.25
2	0.61	0.26	0.60	0.24
4	0.69	0.35	0.66	0.32

TABLE 3. Heritability Estimates with Increasing Number of Readings from Each of Three Tonometers

No. of Readings	GAT		ORA		DCT	
	b^2	Environment	b^2	Environment	b^2	Environment
1	0.56 (0.44-0.65)	0.44 (0.35-0.56)	0.55 (0.44-0.64)	0.45 (0.36-0.56)	0.57 (0.47-0.66)	0.43 (0.34-0.53)
2	0.58 (0.46-0.67)	0.42 (0.33-0.53)	0.61 (0.50-0.69)	0.39 (0.31-0.50)	0.59 (0.48-0.68)	0.41 (0.32-0.52)
4	0.64 (0.55-0.72)	0.36 (0.28-0.45)	0.69 (0.60-0.76)	0.31 (0.24-0.40)	0.67 (0.57-0.74)	0.33 (0.26-0.43)

one's height in the reported group. They concluded that moderate reduction in measurement error (through the use of accurate clinical measurement or multiple self-report measures) increases the effective sample size by 22% and that eliminating the measurement error completely leads to increases in effective sample size of up to 41%. This finding is in agreement with our use of the mean of several measurements in the present study.

All the previous studies that were conducted to determine the heritability of IOP used the readings from only one eye for the analyses.^{5,7,9,10} The eye was selected randomly or the one with the highest IOP was used. In all these studies, except the Beaver Dam Eye Study⁷ for which only one reading was taken, the mean of two or three readings was used for analysis. In an earlier study by Levene et al.,⁸ there is no clear reference to the number of readings used for the analysis. It is fair to assume that if the other studies had used the mean of more readings for their analyses, or the readings from both eyes, they too may have found higher heritability estimates for IOP.

It is essential in the planning of any scientific study to assess how many samples must be collected to have sufficient power to detect the hypothesized effect, especially in genetic studies, in which the costs for genotyping and phenotyping very large samples are high. This study has shown that by increasing the number of readings used and therefore reducing the measurement error, the power calculations to detect linkage increase; which means that the effective sample size used may be reduced, therefore making large genetic studies more manageable in terms of sample size and cost. Taking GAT as an example; to achieve the same power, the sample size required decreased from 7825 to 5538, when the number of readings used increased from one reading only, to the mean of four readings. This reduction in the sample size required amounts to ~30%.

In conclusion, we have demonstrated that by using the mean of several readings from both eyes and therefore reducing the measurement error, the heritability estimate is increased. This will in turn decrease the residual variance, increasing the power to detect linkage in a genome-wide

analysis. Since moderate reduction in measurement error will lead to large increases in effective sample size for linkage analysis, we recommend that researchers take steps to identify possible sources of measurement error and eliminate them. As shown in this study, a simple way of reducing error is by using the mean of multiple readings for analysis. The use of repeated measures in clinical practice may also improve accuracy, and ophthalmologists should consider more than a single measure of IOP when assessing patients.

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TABLE 4. Genetic Power Calculations for Each of Three Tonometers

No. of Readings	GAT	ORA	DCT
1	7825	8127	7527
2	7231	6366	6939
4	5538	4256	4753

The power to detect linkage increased as a result of increased measurements. These values are the predicted sample size required for $\alpha = 0.05$ with a power of 80%. As can be seen, the sample size necessary to detect linkage decreased significantly as the number of readings increased.

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