Application of Advanced Statistics in Ophthalmology

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Statistics is an integral part of research in ophthalmology. The application of appropriate statistical strategies allows clinicians to realize the full potential in analyzing data from paired ocular measurements, longitudinal design, and genome-wide association study (GWAS). The increasing popularity of longitudinal follow-up in either clinical or epidemiologic study demands advanced statistical methodologies. This article describes robust statistical models that can cope with correlated components for both paired-eye data and repeated measurements over time. Also highlight are the statistical challenges and corresponding strategies available for testing multiple hypotheses with paired-eye data in GWAS, which has been the subject of intense interest for the past 5 years within the ophthalmology community in investigating the genetic etiology of eye disorders. (*Invest Ophthalmol Vis Sci.* 2011;52:6059–6065) DOI:10.1167/iovs.10-7108

There are several statistical challenges in ophthalmic research. The nature of ocular measurements of paired eyes poses a long-standing question to ophthalmologists for developing joint inference on paired-eye data.[1,2] The increasing focus on longitudinal designs in clinical trials and observational studies has also compounded the need for statistical methodologies that handle correlated data from repeated sampling across time. Furthermore, the advent of large-scale genetic studies has permitted an unbiased systematic survey of the entire genomic landscape for variants that contribute to the etiology of eye disorders. However, this approach faces the statistical dilemma of testing multiple hypotheses, since a typical genome-wide study queries up to a million variants simultaneously. The appropriate application of statistics in modern ophthalmology research is thus vital in addressing these challenges. In this article, we review problems common to longitudinal and genome-wide surveys of eye-related traits and provide an exposition of proposed solutions.

Paired-Eyes Measurements

Whether data from one eye or both eyes are used depends on the study hypothesis and clinical relevance. For example, visual acuity (VA) in the better eye is commonly used to indicate the degree of visual impairment. Similarly, using the ocular data of the worse eye is an appropriate definition to characterize the status of eye disease for patients eligible for clinical trials. When correlated measurements from both eyes (such as intraocular pressure or refractive error) are available, using the information from only one eye is a statistically simple approach, but may not reflect the true extent of the disease.[3] The rationale behind using one eye (right, left, or randomly chosen) stems from the notion that most ocular measurements are more similar between the eyes of the same individual than between different individuals.[1] However, even though the measurements of both eyes correlated highly, it does not necessary mean that the analysis restricted to only right eyes will yield the same results as those of left eyes. Therefore, cautious interpretation of any discordant results is required. This paired eye problem affects the analysis across different study designs such as case-control, clinical, cross-sectional, and cohort studies. We examined clinical and epidemiologic articles published in IOVS and *Ophthalmology* from January to June 2009 and documented their analytic approaches. Of the 115 papers that are covered in our review, clinical studies exhibited a greater preference to consider all eligible eyes or the affected eye(s) than epidemiologic studies (Table 1A), whereas paired-eye designs are more commonly adopted in clinical trials.[4]

In the paired-eye design, both eyes of the subject are considered as matched case–control data within every subject. The pairing nature in such settings leads to the use of the paired-sample *t*-test and the McNemar test in ophthalmology to assess the differences between paired eyes for numerical or categorical outcomes.[4] Under the assumption that both eyes experience the same exposure within an individual, failure to account for the intrasample correlation between both eyes can overestimate the treatment effect, which leads to an increased likelihood of making a type I error.[1,5] Advanced statistical approaches that are used to perform joint modeling of paired-eye data have been covered in previous reviews.[1,2,6,7] The generalized estimating equation (GEE) is the extension of linear regression within a longitudinal framework where repeated measurements are made within every individual.[8–10] Mixed-effects regression modeling provides a flexible framework for analyzing clustered data with multilevel structures.[9,11,12] Comparisons between GEE modeling and mixed-effects regression on continuous and binary paired-eye data reveal similar performance under most conditions,[6,7,13] although GEE is recognized to be computationally more efficient in handling large datasets with binary or ordinal outcomes.[3]

Longitudinal Follow-up

Longitudinal data arising from either clinical trials or cohort studies allows the progression or natural evolution of a disease to be studied. This is often not achievable with cross-sectional study designs. Data in longitudinal follow-up studies are generally collected in the form of outcomes measured repeatedly or the time until the onset of the disease.[9] The former mainly focuses on modeling the changes in health outcomes over time and on identifying the factors that are associated with the

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Table 1. Categories of Analytic Strategies in Clinical and Epidemiological Papers*

A. Analyses at Subject Level versus Ocular Level†

	Articles *n* (%)	No Correction for Correlation on Paired Eyes‡
Clinical study		
Subject level	24 (20.9)	—
Ocular level	24 (20.9)	10
Epidemiology study		
Subject level	48 (41.8)	—
Ocular level	19 (16.4)	4
Total	115 (100)	

B. Statistical Approaches for Longitudinal Follow-up Study§

	Articles, *n* (%)
t-test/paired t/χ^2/McNemar test	18 (26.1)
Wilcoxon rank sum test	11 (15.9)
Logistical/linear regression	10 (14.5)
Repeated ANOVA	6 (8.7)
Mixed model/GEE	11 (15.9)
Survival based analysis	13 (18.9)
Total	69 (100)

* Summarized from 115 clinical and epidemiological articles published in *IOVS* and *Ophthalmology* from January to June 2009, excluding case reports, noncomparative studies, studies with outcome unrelated to eye measurements, studies on test-retest reliability, and meta-analyses.

† Subject level is the analysis performed with each individual as the unit, and we cannot distinguish whether paired-eye data are available or not. Ocular level is the analysis conducted with each eye or affected eye as the unit.

‡ Studies neither explicitly demonstrate nor mention the correlation adjustment between both eyes in the analysis.

§ Longitudinal studies consist of clinical follow-up and longitudinal observational studies from both prospective and retrospective cohorts. The statistical approaches are considered as those main statistical methods in analyzing the primary outcome.

changes; the latter primarily investigates the factors associated with the risk of event onset. In ophthalmology, inclusion of paired-eye data in a longitudinal study for some or all the study participants complicates the multilevel structure of these data, and the complexity should be recognized before the initiation of the study.

Our literature review suggests that most studies adopted the use of simple analytical methods (see Table 1B), either in a cross-sectional fashion at discrete time points or to measure the changes in a numerical outcome over time. Both approaches transform data from repeated measurements into a single outcome for each subject, where traditional statistical strategies (such as paired *t*-test, χ^2 test and regression modeling) can be used in the analysis. The aggregating of the outcome is appropriate in a variety of situations in ophthalmology, such as measuring the average change of intraocular pressure or quantifying the corneal refractive error before and after surgery for patients with astigmatism. However, it is important to recognize that these strategies do not use all the information that is available for each subject. To obtain a comprehensive understanding of how the outcome changes over time, especially in the presence of a treatment where the efficacy changes with time, it becomes necessary to explicitly model the repeated measurements within each subject. This explicit modeling becomes even more important when an experiment focuses on an eye trait that is liable to exhibit large discordances between the eyes, such as a longitudinal assessment of intraocular pressure progression in individuals with presence of uveitis in affected eye(s). In such a study, the failure to incorporate the correlation between the eyes will bias the statistical inference to assess the eye-specific risk factor on the longitudinal progression of the outcome.

Appropriate analyses of longitudinal data should consider the correlation structure between the repeated measurements. Established methods include repeated measurements analysis of variance (ANOVA),[14–16] GEE,[8–10] and mixed-effects regression model[11,17,18] (Table 2). Repeated-measures ANOVA primarily focuses on balanced data where the same number of repeated observations has been made for every individual. However, this condition is often not fulfilled in observational studies. In an unbalanced design where the number of measurements for each individual may differ, mixed-effects models and GEE are the preferred methods to analyze longitudinal data. In mixed-effects modeling, the joint consideration of fixed and random effects estimates both a subject-specific baseline for the outcome and a subject-specific trend (over time) for the explanatory variables,[11] which allows the extent of interindividual variations to be measured. Random effects can be assumed on any covariate or any cluster of subjects to capture the correlated characteristics in the data; fixed-effects estimates are interpreted as the conditional effects in the presence of the covariates with random effects. In GEE, the "sandwich covariance" effectively estimates the correlation structure between all pairs of observations from the same cluster, yielding robust estimates of the standard errors for the regression coefficients while allowing the marginal treatment effects to be calculated.[14] In the case of nested multilevel structure, GEE considers the cluster at the top in assessing the potentially correlated outcomes.[19] Although both mixed-effect and GEE modeling are commonly used in ophthalmology to handle numerical and discrete outcomes,[11,12] it has been suggested that mixed-effects regression is more efficient in the presence of data with a substantial amount of nonrandom missingness.[9,17]

To illustrate the analytic approaches for fitting longitudinal data with repeated paired-eye measurements, we consider a dataset from the Singapore Cohort Study of the Risk Factors for Myopia (SCORM),[20] where a total of 1979 school children recruited from 1999 to 2001 were followed up longitudinally for the myopia development. For illustration purposes only, our primary interest is whether the school that the child comes from is associated with the students' refractive error measured annually for four consecutive years. Sphere equivalent (SE) measurements (four per eye) are clustered at eye level, and eyes (two per individual) are clustered at subject level. We perform four sets of analyses. First, we fit the repeated-measurement SE using data from both eyes of each participant by mixed-effects model (model 1) and GEE (model 2). For the mixed-effects model, the subject and eye are modeled as random effects in a nested structure, whereas GEE relies on empiric covariance estimates for the subject clusters.[21] Second, we model SE longitudinally using measurements from both eyes by mixed-effects (model 3) and GEE (model 4), but ignore the intereye correlation for each individual. We consider data from the right eye and those from the left eye as independent observations. Third, we fit the repeated measurements of SE average from paired eyes using mixed-effects model (model 5) and GEE (model 6). Fourth, we model SE at the last visit (year 4) for the data of right eye only from each individual, where observations from previous visits and from the left eye are deliberately excluded from this analysis (model 7). Table 3 compares the results of various approaches to modeling longitudinal SCORM dataset.

The first analytic approach offers distinct advantage over others at it correctly models repeated measures and intereye

TABLE 2. Statistical Approaches for Longitudinal Follow-up Study

Approaches	Outcome	Adjust for Correlation: Paired Eyes	Adjust for Correlation: Repeated Measures	Comments
Charting Event Progression				
t-test/ANOVA χ^2 Wilcoxon rank tests	Continuous/discrete	No	No	Straightforward; perform analysis at each time point or use changes as outcome, less powerful due to discarded information; cannot model the time trend or the predicators associated with outcome
Linear/logistical regression	Continuous/binary	No	No	Straightforward; perform analysis at each time point or use changes as outcome; adjust baseline covariates in the model; less powerful if discarding information; cannot model the longitudinal trend
Repeated ANOVA[14]	Continuous	Yes	Yes	Analytically complex; require balanced data design; less robust to missing data; cannot model individual trend
Mixed-effects model[11,17]	Continuous/binary/count	Yes	Yes	Statistically powerful; analytically complex; can model both fixed and random effects; flexible framework in specifying parameter distribution; capable of handling unbalanced data
GEE[10]	Continuous/binary/count	Yes	Yes	Statistically powerful; analytically complex; capable of handling unbalanced data; model marginal effects; less powerful in handling missing data
Charting Event Onset	***Time to Event***			
Kaplan-Meier	Continuous	No	NA	Straightforward; estimate the survival rates
Log rank test	Continuous	No	NA	Simple nonparametric approach to compare the rates; unable to adjust covariates
Proportional Cox model	Continuous	No	NA	Quantify effects of covariates on the survival time; compare the rates by groups
Frailty model[26]	Continuous	Yes	NA	Analytically complex; capable of modeling correlated time to event data; flexible framework for random effects
Marginal model[27]	Continuous	Yes	NA	Analytically complex; capable of modeling correlated time to event data; robust to time-dependent covariates; estimate marginal effects

NA, not available.

correlations simultaneously. We observe a significant difference of refractive error between two schools, where the difference varies linearly with time. The statistical significances for the school effect agree between models 1 and 2, whereas the estimated effect sizes are moderately different as GEE calculates the population-averaged effect. In the second scenario (models 3 and 4), longitudinal analysis at ocular level without allowing for intereye correlation results in artificially narrowed interval estimates of the school effect, even when the point estimate of the effect remains unbiased. Failure to account for intereye correlation results in an inflation of the level of statistical evidence. In the third situation, averaging the responses from both eyes results in a larger standard error of the school effect. This is particularly relevant when missing responses are generated as measurements available for only one eye or when ocular measurements are weakly correlated between the two eyes. In this setting, the main effect of school remains significant but is less significant than that from the first set of analysis. In the fourth scenario which considers right eye data only from the last visit, the effect of school is less significant compared to the reportedly significant results. This suggests that the use of limited or partial data can compromise the statistical power. It is also important to note that reducing longitudinal data to cross-sectional fashion in the fourth scenario does not yield any information on the trend or the school effect varying with time, which is often of interest in longitudinal studies.

For the survival-based analysis used in longitudinal follow-up studies, researchers are most interested in the occurrence of the event and time to event onset. Established statistical approaches include the use of the Kaplan-Meier curve, log rank statistics, and Cox proportional hazard modeling.[22–24] If paired-eye data are of interest in the study, time-to-event is not independent at the ocular level. To fit correlated survival data, frailty model, multilevel survival or marginal models are commonly adopted in the medical community.[25–27] The application of such advanced models in ophthalmic research is worth further exploration, but is beyond the scope of this article.

GENOME-WIDE ASSOCIATION STUDY

Genetic studies are conducted to identify the hereditary nature of diseases and traits, primarily relying on the comparison of genetic variation between individuals with differential expression for the trait of interest. A typical genome-wide association study (GWAS) surveys between 500,000 and 1,000,000 single-nucleotide polymorphisms (SNPs) across the entire human genome simultaneously, and such genome-wide designs have replaced candidate gene studies as the preferred strategy to study the genetic etiology of complex human traits,[28,29] including eye disorders.[30–39] Cochran-Armitage trend test, χ^2 test and logistic regression model are largely used in the case–control design to study the overrepresentation of the mutated allele in cases versus controls.[40] In family-based studies, we measure the excess transmission of any allele from heterozygous parents to affected offsprings under the condition of Mendel's law.[41] Furthermore, the incorporation of longitudinal information such as modeling time to event and repeated measurements will add merit to GWAS.[42]

Testing multiple hypotheses simultaneously to draw correct statistical inference is the most challenging aspect in GWAS. It is now common to assay a million variants in a GWAS, and this effectively constitutes a million hypothesis tests. A conven-

Table 3. Results of Analyzing Repeated Sphere Equivalent in a Longitudinal Study Using Different Analytic Approaches

	Whole Data Analysis								Partial Data Analysis					
	Longitudinal Data: Account for Intereye Correlation				Longitudinal Data: Ignore Intereye Correlation				Longitudinal Data: Both-Eyes Average				Cross-Sectional Data	
	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
Intercept	−0.29 (0.42)	0.492	−0.50 (0.48)	0.296	−0.27 (0.32)	0.399	−0.48 (0.35)	0.167	−0.22 (0.49)	0.661	−0.56 (0.56)	0.316	−1.93 (0.63)	0.003
School														
1		Referent		Referent		Referent		Referent		Referent		Referent		Referent
2	0.78 (0.29)	0.009	0.87 (0.30)	0.003	0.75 (0.22)	0.001	0.87 (0.22)	<0.0001	0.73 (0.34)	0.033	0.88 (0.35)	0.013	1.26 (0.61)	0.040
Time, y														
1st		Referent		Referent		Referent		Referent		Referent		Referent		
2nd	−0.70 (0.04)	<0.0001	−0.64 (0.06)	<0.0001	−0.70 (0.04)	<0.0001	−0.64 (0.05)	<0.0001	−0.70 (0.06)	<0.0001	−0.64 (0.07)	<0.0001		
3rd	−1.27 (0.05)	<0.0001	−1.27 (0.13)	<0.0001	−1.28 (0.05)	<0.0001	−1.27 (0.10)	<0.0001	−1.29 (0.07)	<0.0001	−1.35 (0.15)	<0.0001		
4th	−1.73 (0.06)	<0.0001	−1.71 (0.18)	<0.0001	−1.74 (0.06)	<0.0001	−1.71 (0.14)	<0.0001	−1.74 (0.10)	<0.0001	−1.80 (0.22)	<0.0001		
School* time	0.18 (0.02)	<0.0001	0.15 (0.07)	0.030	0.18 (0.02)	<0.0001	0.15 (0.05)	0.005	0.17 (0.08)	<0.0001	0.17 (0.08)	0.037		
Age, y														
7		Referent		Referent		Referent		Referent		Referent		Referent		Referent
8	0.32 (0.27)	0.235	0.38 (0.27)	0.153	0.30 (0.20)	0.149	0.38 (0.20)	0.054	0.27 (0.32)	0.392	0.41 (0.32)	0.197	0.47 (0.41)	0.250
9	−0.90 (0.30)	0.004	−0.92 (0.36)	0.010	−0.88 (0.23)	<0.0001	−0.92 (0.27)	0.001	−0.85 (0.34)	0.014	−0.93 (0.39)	0.018	−0.88 (0.45)	0.051
Sex														
Male		Referent		Referent		Referent		Referent		Referent		Referent		Referent
Female	0.25 (0.23)	0.278	0.18 (0.24)	0.474	0.20 (0.17)	0.253	0.18 (0.18)	0.341	0.14 (0.27)	0.607	0.06 (0.28)	0.821	0.12 (0.34)	0.733
Race														
Non-Chinese		Referent		Referent		Referent		Referent		Referent		Referent		Referent
Chinese	−0.78 (0.28)	0.005	−0.60 (0.30)	0.041	−0.76 (0.21)	<0.001	−0.60 (0.22)	0.006	−0.77 (0.33)	0.018	−0.43 (0.35)	0.214	−0.83 (0.42)	0.049
Books read per week														
≤2		Referent		Referent		Referent		Referent		Referent		Referent		Referent
>2	−0.20 (0.24)	0.401	−0.15 (0.26)	0.563	−0.14 (0.18)	0.447	−0.15 (0.19)	0.442	−0.05 (0.28)	0.849	−0.11 (0.29)	0.700	−0.13 (0.36)	0.730

$N = 300$. Data are from the Singapore Cohort Study of the Risk factors for Myopia (SCORM). We analyzed data of randomly chosen 300 children in school 1 or 2 with at least one visit (534 eyes in total and 1837 observations for SE measurements). β, coefficient (effect size) of the covariates; SE, standard error of β. Full data analysis on repeated measurements of SE, adjusting for intereye correlation using mixed-effects (model 1) or GEE (model 2). Full data analysis on repeated measurements of SE ignoring intereye correlation, using mixed-effects (model 3) and GEE (model 4). Reduced data analysis on repeated measurements for average SE using mixed-effects (model 5) and GEE (model 6); reduced data analysis on SE measurements of the right eye at year 4, using linear regression (model 7). The programs, written in free-software R (URL http://www.R-project.org), are provided in the Supplementary Material (http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-7108/-/DCSupplemental).

tional significance threshold of 5% is thus expected to artifactually identify 5000 markers that are "correlated" to the trait. To address this problem with multiple testing, geneticists have adopted a stringent statistical significance level of 5×10^{-8}, commonly defined as genome-wide significance, the benchmark for evaluating the fidelity of the association signal at each marker.[40] Replication is considered the gold standard in GWAS publications.[43] The identification of candidate genetic loci for replication is mainly driven by the level of statistical evidence from single-marker association tests (either the *P* value or the Bayes factor).[40,44] More advanced approaches, for example, pathway-based analyses and epistasis tests, have also been proposed to prioritize genetic markers for further downstream functional evaluation. These analytic strategies have been covered comprehensively in previous reviews.[45,46]

In gene mapping, phenotypes are usually classified into two broad types: qualitative (or binary) and quantitative (or continuous) traits. Dichotomous traits have been featured in GWAS for age-related macular degeneration (AMD),[34,35] primary open-angle glaucoma (POAG),[31,39] cataract,[37] and high myopia.[36,38] The affected individuals are usually classified on the basis of diagnosis from the worse eye or both eyes, whereas controls exhibit no sign of syndrome for both eyes. Although assessing the binary outcome is more directly relevant to clinical application, quantitative traits (endophenotypes or intermediate traits) underlying diseases are also valuable in the dissection of the genetic architecture, as they take the full-spectrum measures into account. For instance, central corneal thickness (CCT) and cup-to-disc ratio (CDR) are presented as quantitative endophenotypes of open-angle glaucoma (PORG).[47] Mapping genes for CCT[48–50] and CDR[51,52] in GWAS would shed light on the joint genetic etiology of PORG.

Often, the primary interest in ophthalmic genetic studies for quantitative trait is to locate shared genetic loci that exert effects on both eyes,[53–55] as the physiological mechanism underlying intereye difference of phenotypic abnormalities remains elusive and inadequately understood. Therefore, for quantitative traits collected from both eyes, an immediate question is whether the analyses should be performed on data from one eye or both eyes. In seven GWAS papers on eye-related QTL that have been published to date (http://www.genome.gov/gwastudies), the analytic strategies varied from the use of the right eye,[49,50,52] to a randomly chosen eye,[51] to the averaged measurement from both eyes.[32,33,48] Conducting analysis to one eye alone is a simple approach to avoid statistical model complexity. However, using partial data of one eye only may be statistical insufficient. Averaging ocular measurements between both eyes has been suggested to yield higher heterogeneity estimates than using information from one eye only and therefore tends to have more power in genetic studies.[56] Using averaged ocular measurements therefore has been the convention in linkage study for quantitative trait in the myopia genetics research community.[57–60] In a few scenarios in which the traits may be moderately or weakly correlated between the two eyes, however,[1] neither the use of data from one eye nor an average from both is appropriate, because of the negligence of phenotypic dissimilarity.

A wide array of statistical approaches has emerged recently for the detection of the pleiotropic genetic factors contributing to multiple correlated traits, which could also be applied to paired-eye data (Table 4). Simultaneous consideration of all correlated phenotypes is shown to be statistically powered to exploit the pleiotropic genetic effects over the univariate analysis.[61–64] The first approach is to combine dependent test statistics or estimators from the univariate analyses for a global assessment on association.[61,65–67] In brief, GWAS tests are conducted for the two eyes separately. The two test statistics from both eyes (for example, z scores) are combined subsequently in a linear form weighted by the covariance matrix estimates.[61,67] Correcting for twice the number of markers is not relevant here, since only one global test is performed for each marker, using the combined statistics. This simple approach does not rely on a complicated model assumption. The second approach is to transform multiple traits to an optimal single phenotype with enhanced heritability, and one such example is principle component analysis.[62,68] This dimension-reduction technique involves intensive computation; thus, the application in paired-eye data may not be straightforward. The third one is model-based joint analysis of bivariate traits, including GEE,[63,69–71] mixed-effects,[64,72] and tree-based regression,[73] et cetera. Of these, the GEE model is the most statistically efficient in performing bivariate association tests.[63,71] To date, few statistical software programs incorporating model-based joint analyses on bivariate traits are available[74]; much more effort should be devoted to this area.

Accumulated evidence suggests that most of the GWASs are underpowered, especially for the common variants with small-effect sizes and the associated SNPs generally explain little genetic variation.[75] Meta-analysis provides a robust approach to enhance statistical power and effective sample size by pooling evidence from multiple independent association studies.[76,77] Application of meta-analysis in ophthalmology

Table 4. Summary of Analytic Approaches for Quantitative Trait of Both-Eyes Data in GWAS

Approaches	Comments
Data from One Eye	
Either eye or a randomized eye	Simple; less powerful if the correlation between the two traits is low
Data from Both Eyes	
Transform bivariate traits to a single trait average measurements	Simple and efficient; statistically less efficient if the correlation between bivariate trait and missing data present on either eye is low.
Principle components analysis[62,68]	Statistically powerful; complex; reduce the phenotypes to a single trait; computational intensive.
Combining univariate test statistics[61]	Simple and powerful; capable of handling paired-eye traits not highly correlated; robust for partially missing trait values; non-parametric.
Model-Based Approaches	
GEE[63,69–71,74]	Statistically powerful; robust for various correlation structures; efficient on both normal and nonnormal traits; complex
Mixed-effect model[69,70]	Statistically powerful; complex; robust for various correlation structures of multiple traits; computational intensive
Tree-based regression[73]	Analytically complex; capable of assessing multiloci association test; computation extremely intensive

has become a standard practice to identify genetic polymorphisms that are associated with eye disorders.[32,33,49–52] If the individual GWAS is conducted with different genotyping platforms, the meta-analysis strategy could use only a small subset of overlapping markers. One way to address this problem is imputation-based meta-analysis. It provides a powerful framework for the assessment of the complete array of genetic variants (most of which are untyped). Step-by-step guidelines and techniques for performing imputation-based genome-wide meta-analysis were reviewed by de Bakker et al.[77] In meta-analysis, using homogeneous populations with the similar genetic background, phenotype definition, and sample ascertainment will increase the likelihood of identifying the genuine genetic association.[78] In the presence of heterogeneity across different studies, carefully examining the potential factors that cause heterogeneity is crucial to enhance the credibility of the combined evidence.

Conclusions

Adopting appropriate statistical methods will permit us to explore the full potential in the analysis of the data and make valid statistical inference. The simple statistical approach commonly used in longitudinal studies by using reduced data in ophthalmology may be useful in some scenarios, but is insufficient to explicitly model the trend of the treatment effects or the longitudinal change of the outcome. In addition, if paired-eye data are involved in longitudinal studies, lack of adjustment for the correlation between the eyes violates the underlying assumptions of independent observations. From a methodological point of view, both GEE and mixed-effects modeling play an increasingly important role in analyzing longitudinal repeated measurements and paired-eye data simultaneously. In GWAS, the statistical challenges raised for ocular traits center on multiple hypothesis testing and analyzing paired-eye data appropriately. Different approaches have been used for analyzing paired-eye data under various GWAS conditions, and the best strategy should be considered for all the factors at the study initiation. Understanding the strengths and weaknesses of the statistical methods enhances our ability to correctly interpret the GWAS and differentiate robust findings from spurious ones; this is especially vital, given the oncoming flood of GWAS data in the genomic era.

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