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Ophthalmic Phenotypes and the Representativeness of Twin Data for the General Population

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Abstract

Purpose: To compare the distributional parameters for a series of ocular biometric traits between twins and their singleton siblings in order to evaluate the generalizability of twin data as used in heritability analyses to the general population.

Methods: A series of birth, anthropometric and 13 ocular biometric traits were selected for analysis: inter-pupillary distance (IPD), visual acuity (logMAR), spherical equivalent refractive error, corneal curvature, axial length, anterior chamber depth (ACD), central corneal thickness (CCT), intra-ocular pressure (IOP), optic disc, cup and rim areas, and measures of retinal vessel caliber; central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). Structural equation modelling was used to test the assumption that the means and variances for each trait did not differ between twins and their siblings.

Results: Significant differences in log-likelihood for birthweight and gestational age were observed between twins and siblings, with the latter being both heavier and closer to be full-term at birth. Siblings were also found to have larger IPD and axial length, and better visual acuity compared to their twin counterparts. Refractive error, corneal curvature, ACD, CCT, optic disc parameters and retinal vascular calibers did not differ significantly between the two groups.

Conclusions: Twins are representative of the general population for some but not all measures of ocular biometry. Consequently, care should be taken when extrapolating twin data for these traits in heritability and other genetic studies. Birthweight differences between twins and siblings do not appear to account for the differences in ocular biometry observed in this study.

Introduction

The classic twin study represents one of several investigative methodologies in genetic epidemiology designed to quantify the relative contribution of genes and environment to biological traits and disease. The interpretation of estimates of heritability from twin data is based on implicit assumptions of twin-singleton trait comparability, which influences the extent to which such results may be considered applicable to the general non-twin population. In addition to non-random sampling, this reflects a frequent criticism of the twin method in its purported non-generalizability to singletons due to differences in developmental (e.g. intrauterine, family) environment between the two groups. Certainly, it is well accepted that on average, twins are more likely to be born premature and have lower birthweights than singleton pregnancies.[1, 2] The representativeness of twins for the general population for a trait found to be associated with such neonatal outcomes therefore requires cautious interpretation. Further to this, the "Developmental Origins of Health and Disease (DOHaD)", or "Barker hypothesis" is illustrative in postulating the epidemiological evidence supporting an inverse association between birthweight and adult cardiovascular disease is more a causal effect governed by adverse prenatal exposures.[3] In a recent twin study, however, de Geus et al. found no evidence in favour of this theory, with no difference in blood pressure observed between twins and their siblings.[4] In fact it is likely that common genetic factors may play a mediating role in this relationship.[5]

Differences in placentation between monozygotic (MZ) and dizygotic (DZ) twins also have the potential to impact the validity of extrapolation from twin findings. While all DZ twins have separate placentas (dichorionic), approximately 60% of MZ pregnancies are monochorionic, which carries an increased risk of vascular abnormalities during gestation.

Asymmetric fetal sharing of the common placenta often result in birthweight differences between twins and may subsequently affect postnatal growth.[6] Given that placentation data are infrequently collected, another concern relates to potential disparities between monochorionic and dichorionic MZ trait correlations, which may challenge the most fundamental assumptions of the classic twin method. Of all twin types, dichorionic MZ or DZ twins carry the lowest risk of perinatal mortality at approximately 9%; however, this still remains greater than that observed with singleton pregnancies.[7]

There is a paucity of information in the literature considering the generalizability of twin data. Silventoinen et al. examined the heritability of body size and muscle strength in a prodigious cohort of Swedish males and found that differences between singletons and twins can persist into adult life with twins being shorter, lighter and demonstrating lower muscle strength than singletons.[8] In addition, singletons showed more variation in weight and strength measures compared to twins with known zygosity, probably resulting from selection bias due to non-participation. Subsequently, previous heritability estimates for these anthropometric parameters may have been spuriously inflated. The use of unrelated singletons can complicate the statistical modelling given the lack of matching for genetic and family environment. Utilising siblings of twins ("Extended twin design") largely overcomes this difficulty while increasing the statistical power of the study, however, differences in trait values between these groups would obviously negate this advantage.

We conducted a large twin study to evaluate genetic and environmental determinants of ocular biometry and eye disease with a particular interest in glaucoma and myopia endophenotypes.[9] In addition to determining heritability estimates, we performed microsatellite linkage[10, 11] and a GWAS (genome-wide association study)[12, 13] on this cohort.

Where available, siblings have been examined as part of the Brisbane Adolescent Twin Study under an extended family study design. Little information is available regarding the generalizability of twin data for ocular biometric traits. To date there has been only one study specifically investigating the representativeness of twins to the general population and this was for refractive error.[14] We had previously shown no difference in retinal blood vessel diameter measurements between the two groups but wished to extend this to all the major biometric measures.

Materials and Methods

Participants

Twin pairs were identified from two cohorts, the Twins Eye Study in Tasmania (TEST),[9] and the Australian Twins Eye Study (ATES).[15] The relevant ethics committees of the Royal Victorian Eye and Ear Hospital, University of Tasmania and Queensland Institute of Medical Research approved the study. The study adhered to the tenets of the Declaration of Helsinki. Data were available for 2053 individuals, including 929 twin pairs (381 MZ, 548 DZ) and 195 non-twin siblings (102 siblings of MZ pairs and 93 siblings of DZ pairs). The vast majority of participants (> 95%) were Caucasian in origin. Subjects who had ocular or systemic disease preventing a complete ophthalmic examination were subsequently excluded from the analysis.

Traits Examined

All twins and siblings participating in the study underwent a comprehensive clinical examination that included anterior segment examination, corneal pachymetry, intraocular

pressure (IOP) measurement, auto-refraction, and a mydriatic optic disc assessment. The same measurement methods and instruments were utilised in both groups. Simultaneous stereoscopic optic disc photographs were taken with a fundus camera (3-Dx/F; Nidek, Gamagori, Japan) on 35-mm slide film (Ektachrome; Eastman Kodak, Melbourne, Australia). For all twin pairs, zygosity was confirmed by DNA analysis of short tandem-repeat (STR) and subsequent GWAS.

A series of 13 ocular biometric traits were selected for analysis; inter-pupillary distance (IPD), visual acuity (logMAR), spherical equivalent refractive error, corneal curvature, axial length, anterior chamber depth (ACD), central corneal thickness (CCT), IOP, optic disc, cup and rim areas, and measures of retinal vascular caliber, central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). The mean values of right and left eyes were used in the analysis. Full details of trait examination protocols have been described previously.[9]

Statistical Analysis

Data management and statistical tests were performed in the R statistical environment.[16] Descriptive statistics were calculated for each trait in addition to measures of spread, including kurtosis and skewness. Distributions were tested for normality with the Shapiro-Wilks test and were considered significantly different from normal for $p < 0.05$. Boxplots were constructed for each trait for twin (stratified by zygosity) and sibling data. Unless otherwise indicated data are presented as mean $\pm$ standard deviation (SD).

A fundamental assumption of genetic modelling in the classic twin design is that the means and variances of the traits are not significantly different by birth order and zygosity. The discovery of such differences may be indicative of ascertainment bias, measurement

error, or natural variation. On the other hand, if siblings can be shown to have the same mean-variance structure as their twin counterparts, there is sound reasoning in asserting the generalizability of twin data for that trait (in the context of a heritability study, the equal environment assumption should also be satisfied).[17] An elegant method for assessing these distributional moments is to use Structural Equation Modelling based on maximum likelihood, the primary advantage being that the technique allows parameter estimation rather than simple parameter calculation.[18]

Data for each trait were z-transformed and winsorized such that the extreme 0.3% of values in this study (i.e. those exceeding $\pm$ 3 SDs from the mean) were replaced with $\pm$ 3. Winsorization is a common statistical technique for dealing with data outliers, such that distributional tail values are constrained to some pre-specified percentile. Data were then subset into eight groups based on zygosity and sex: monozygotic females (MZF), monozygotic males (MZM), dizygotic females (DZF), dizygotic males (DZM), dizygotic opposite sex pairs with females first born (DZFM) and males first born (DZMF), female siblings (Sib F) and male siblings (Sib M). OpenMx software[19] was utilised to test the assumption that the means and variances for each trait did not differ between individuals within and across groups. In the first instance a saturated (control) model was fitted to the raw data and all parameters freely estimated. Twelve univariate, nested models were subsequently fitted in a stepwise fashion, with each applying additional parameter constraints compared to the previous model (Table 1). In the first nested model, the intra-pair twin means were constrained to be equal within same sex groups (i.e. MZFt1=MZFt2, MZMt1=MZMt2, DZFt1=DZFt2 and DZMt1=DZMt2) to examine birth order effects. In the second model, equality constraints were also placed on the means across same sex groups

(i.e. MZF=DZF and MZM=DZM) to test zygosity effects. Model 3a and 3b examine differences between members of opposite and same sex twin pairs; 3a equates means within females and males across opposite sex pairs (i.e. DZFMt1=DZMFt2 and DZFMt2=DZMFt1) and 3b additionally across same sex pairs (i.e. MZF=DZF=DZFMt1=DZMFt2 and MZM=DZM=DZFMt2=DZMFt1). The fourth model (to test twin-sibling differences) further constrains the means of twins to be the same as their siblings within females and males (i.e. MZF=DZF=DZFMt1=DZMFt2=Sib F and MZM=DZM=DZFMt2=DZMFt1=Sib M). The final means model contains only a mean for males and a mean for females and are subsequently equated to produce one mean for the cohort. The same series of models are then retested whereby variances are constrained instead of means.

Each of the nested submodels was then compared to the saturated model by hierachical χ^2 tests. Twice the difference between log-likelihood values of the nested and saturated models is asymptotically distributed as χ^2 with degrees of freedom (df) equal to the difference in parameters being estimated. The submodel with the lowest difference in log-likelihood is considered to fit best and reflects model parsimony (fewest number of freely estimated parameters) indicated by a non-significant difference between the nested and saturated fits.[20]

All anthropometric and ocular traits were adjusted for covariate effects in this study. The effect of birthweight on each trait was investigated by comparing the saturated model to an equivalent model with this parameter removed. The difference in log-likelihood between the two models therefore provides an indication of whether birthweight may be associated with the phenotype under examination. If the statistical fit deteriorated (i.e. significant difference in log-likelihood), birthweight was retained as a covariate in

subsequent hypothesis testing, otherwise it was eliminated. Additionally, age, age^2, sex*age and sex*age^2 were modelled as fixed effects (regressed from the mean) for each phenotype. The inclusion of age as a quadratic covariate was to allow for non-linear age effects, and was considered important as our cohort displayed large age variation. The addition of sex interactions are necessary as there are no *a prio*ri reasons to expect the influence of age to be the same across sex.

OpenMx does not permit missing covariate data, thus incomplete data (birthweight and gestation data only) for a subject were replaced with the mean of the entire cohort. To minimise Type I error resulting from the large number of tests performed (219), a Bonferroni correction was applied (0.05/219) with statistical significance defined as $p \leq 0.00023$. Significant twin-sibling differences were described by Cohen's d, an effect size invariant to scaling that is measured in SD units.[21] Cohen's d is calculated as follows:

$d = (\mu_{Sib} - \mu_{Twin})/\sigma$

where μ_{Sib} is the mean of sibling group, μ_{Twin} is the mean of the twin group and σ is the pooled standard deviation. Cohen proposed that d values of 0.2, 0.5, and 0.8 represent small, medium, and large effect sizes respectively.

Results

Descriptive statistics for all participants for age, birthweight, gestation, anthropometric and ocular biometric traits investigated are shown in Table 2. The mean $\pm$ SD age across all participants was 20.6 $\pm$ 11.8 years (range, 5-90 years). Figure 1a shows a boxplot of differences in age between twins and siblings. The distribution of age was positively skewed with MZ twins on average two years older than their DZ counterparts. Birthweight and gestation data were available for 82% and 89% of all participants,

respectively. The average birthweight was 2.56 kg and gestation 37 weeks; however, as discussed this depends significantly on the type of pregnancy. The boxplot for birthweight (Figure 1b) shows the expected pattern of lighter MZ twins (2.4 $\pm$ 0.63 kg) compared to DZ twins (2.54 $\pm$ 0.56 kg) and siblings (3.43 $\pm$ 0.59 kg). Gestation period (Figure 1c) follows a similar trend with MZ twins (36.5 $\pm$ 3.22 weeks) being born earlier than DZ twins (37 $\pm$ 2.83 weeks) and siblings (39.7 $\pm$ 1.54 weeks). The boxplots for adult height (Figure 1d) and weight (Figure 1e) are similar between subgroups.

Distribution of Ocular Biometry

The mean IPD of all participants was approximately 61 mm. On average, siblings were noted to have IPDs 2.3 mm larger (63 mm) than twins of either zygosity (Figure 2a). The distributions for visual acuity and spherical equivalent refractive error were the most kurtotic of all examined. Siblings had lower average measures on both visual acuity (i.e. better vision) (Figure 2b) and refractive error (Figure 2c) (-0.52 D) compared to MZ (-0.26 D) or DZ twins (-0.04). Axial length (Figure 2e) displayed a complementary trend to refractive error, with siblings having longer eyes (23.48 mm) than MZ (23.22 mm) and DZ (23.14 mm) twins. Similarly, siblings were observed to have deeper ACDs (Figure 2f) (3.69 mm) and higher IOPs (Figure 2h) (16.6 mmHg) than either MZ (3.62 mm, 16.2 mmHg) or DZ (3.62 mm, 15.5 mmHg) twins. For the remaining traits, twins and siblings appear similar in their distributions for corneal curvature (Figure 2d), CCT (Figure 2g), optic disc (Figure 2i), cup (Figure 2j) and rim (Figure 2k) areas and for both measures of retinal vascular caliber, CRAE (Figure 2l) and CRVE (Figure 2m). For all traits, the Shapiro-Wilks test was significant for distributional departure from normality.

Equability of Means and Variances

The influence of twin order, zygosity, sex and twin-sibling differences for each of the traits examined is shown in Table 3. When we equated the means and variances for birthweight across twins and siblings ($H4_{M,V}$), significant differences in log likelihood were observed compared to the saturated model allowing freely estimated parameters. Thus, as expected, differences exist in birthweight between the two groups. Poor model fits were similarly noted for gestation period when attempting to constrain twin-sibling group means. Adult height and weight was equivocal between subgroups; however, significant sex differences were observed ($H5_M$) with males both taller and heavier than females.

Birthweight was found to be associated with adult height, IPD, corneal curvature, axial length and CRAE, and was subsequently retained as a covariate in hypothesis testing for these traits. Of the ocular biometric traits, poor model fits were predominantly confined to hypotheses concerning means across zygosity ($H2_M$) and twin-sibling groups ($H4_M$). Significant differences between twins and siblings were found for IPD (Cohen's d 0.63), visual acuity (Cohen's d 0.42), axial length (Cohen's d 0.29) and IOP (Cohen's d 0.24). When body height was included as an additional covariate for the analysis of axial length, twin-sibling differences in the parameter were no longer significant. The distributional differences described above for refractive error and ACD were not significant in model testing. Corneal curvature, CCT, optic disc and retinal vascular calibers did not differ significantly between the two groups. Additionally, a highly significant sex difference ($H5a_M$) was noted for IPD, with larger mean values for males compared to females. Differences between members of opposite and same sex pairs ($H3a_{M,V}$ and $H3b_{M,V}$) were generally negligible.

Discussion

The search for genetic variants directly associated with or causing disease has taken on renewed vigor with the recent advent of high-throughput molecular genotyping technologies. Justification for such studies usually begins with validation that the trait under investigation has a heritable component. A commonly misunderstood concept; heritability refers to the proportion of phenotypic variation of a trait in the population studied that is due to genetic factors (the interested reader is referred to a recent review article for a more detailed treatment of heritability and related studies in the field of ophthalmology).[22] Given the majority of ophthalmic heritability studies are based on twin data,[22] a reasonable enquiry relates to the generalizability of the results to the population at large. In this study we characterised the distributions of a series of birth, anthropometric and 13 ocular biometric traits in a large cohort of twins and their siblings. Importantly, the assumption of twin representativeness for the general population was tested by comparing distributional parameters between the two groups.

Considering birth parameters, we confirm the previously reported differences in gestation and birthweight, with siblings more likely to be born full term and to be heavier than twin neonates. A confounding issue regarding differences of gestation duration relates to high prevalence rates of induced or planned cesarean sections in multiple births. Given that twins also have a greater risk of perinatal mortality (labor and delivery complications) than singletons,[23] increased intervention to deliver twins earlier in the third trimester has become common.[24] Our results for adult height and weight twin-sibling trends are similarly in accordance with other published data. In a comparison of female twins from the UK Twin Registry and unrelated singletons, Andrew et al. found DZ twins were on average 600 g

lighter than the singleton cohort, and MZ twins were consistently lighter than their DZ counterparts across all age strata.[25] With regards to height, anthropometric data for participants in the Netherlands Twin Registry found twins and siblings attained similar height in adulthood, but siblings were heavier than twins and interestingly also had increased weight and body-mass index compared to population-based data from national reference growth charts.[26] Although our data failed to show such an association, it's tenable that this disparity is related to differences in birthweight, given the documented positive correlation between neonatal and adult weights.[27]

In relation to ocular biometry, siblings seem to have better visual acuity, longer eyes and greater IPDs than twins. Comparative twin-sibling data for the ocular parameters investigated in this study are scant; however, several published reports have examined associations with birthweight and gestational age largely in the context of retinopathy of prematurity (ROP). In one cohort of low birthweight (< 1700 g) participants born with or without ROP and subsequently examined in adolescence, O'Connor et al. observed higher prevalence of visual impairment compared to full-term infants,[28] with similar findings reported in other studies.[29, 30] The pathophysiology underlying maldevelopment of visual function in preterm infants is poorly understood. Fielder postulated that exposure of immature tissues to environmental illumination may be associated with subtle ocular morbidities including mild vision reduction and arrested eye growth.[31] The birthweights of newborns in many of these studies were very low and are unlikely to be directly comparable with that of our participants, but they may be indicative of potential trends.

Our finding of larger eyes and IPDs in full-term siblings is in accordance with previously published data. In one series of children who developed ROP, axial lengths were

shorter and corneal curvatures larger than in preterm controls delivered without the condition.[32] Cook et al. observed similar results for axial length and corneal curvature, in addition to shallower anterior chambers in low birthweight infants compared to full-term controls.[33] Low birthweight has also been associated with smaller IPDs.[34] Taken together, these are not unexpected findings, given that birth size naturally moderates head size and associated oculometric parameters.[35] There also appears to be a pleiotropic genetic effect for axial length and anterior chamber depth with twin data suggesting that shared genes are responsible for the phenotypic correlations noted in one study.[36] Additionally, recent work by Zhang et al, has shown that axial length is associated with height and that covariation of these parameters is also driven by shared genes.[37] On this basis we re-analysed axial length including height as an additional covariate; the difference in log likelihood ($H4_M$) was 15.31 and no longer significant. Thus, twin-sibling differences in axial length may be explained in part by differences in body height. It may be prudent to adjust for this anthropometric trait in further research investigating the genetic characteristics of axial length that employ twin and non-twin subjects.

The difference in refractive error between twins and siblings in our study was not significant in model testing. However, the longer axial length observed in siblings concurs with them being more myopic, although interestingly we did not observe compensatory disparities in corneal curvature as others have noted.[32, 33] Our finding of equivocal refractive error is consistent with that reported in the literature. O'Connor et al. noted that preterm children were at higher risk for the development of all forms of refractive error.[38] In another cohort of twins, triplets and singletons, no significant differences in the prevalence of refractive error between multiples and singletons was detected.[39] A previous study

investigating the representative of twins for the ocular parameter, refractive error, did not show any significant difference between twins and singletons for myopia, although small differences were observed for hyperopia and astigmatism.[14]

The distributions of IOP among twins and siblings in this study are hard to explain and may reflect the difficulty in obtaining accurate IOP measurements based on a single reading.[40] While the effect size describing the difference between the two groups was small (Cohen's d 0.24), there are no physiological grounds for the observed variation.

Our results showed no differences between twins and siblings for a number of ocular traits including: corneal curvature, CCT, optic disc, cup and rim area and retinal vessel vascular calibers. A recent study investigating the association between birthweight and optic nerve head parameters did find low birthweight was associated with smaller disc areas, larger cup areas and larger cup/disc ratios in children aged 12 years.[41] While our data did not reflect this trend, the possibility that fetal growth restriction may affect optic disc parameters and subsequent long-term glaucoma risk bears consideration. Indeed, using the same cohort described in this paper, Sun et al. found that narrower retinal arteriole caliber in young twins were associated with smaller birth size, thus adding further support for the DOHaD hypothesis.[42]

This work involves multiple comparisons and p values were adjusted accordingly. A more conservative α level was selected as approximately 110 significant results were initially found at $\alpha = 0.05$ when about 19 would be expected to be due to Type I errors. A limitation of this study therefore relates to decreased power to detect differences, particularly for trait differences of small magnitude. For example, power to detect an effect size difference of 0.5 is 99% compared to 15% for an effect size of 0.2, given $\alpha = 0.00023$.

However, a statistically significant finding may not translate to a clinically relevant difference; our goal was to ensure moderate to larger effect sizes were detectable in the traits examined and we are confident this is the case in the current study.

In conclusion, twins are representative of the general population for many, but not all measures of ocular biometry. Thus, in pooling data for genetic studies, consideration needs to be given to traits that may differ and the generalizability of twin-related findings should be reviewed. One potential method may be to account for twin data by incorporating twin status as a covariate in population-based analyses such as GWAS. Furthermore, birthweight differences between siblings and twins do not appear to account for the differences in ocular biometry measures observed in this study.

References

1. Min SJ, Luke B, Gillespie B et al. Birth weight references for twins. Am J Obstet Gynecol. 2000;182:1250-1257

2. Kiely JL. The epidemiology of perinatal mortality in multiple births. Bull N Y Acad Med. 1990;66:618-637

3. Barker DJ. In utero programming of chronic disease. Clin Sci. 1998;95:115-128

4. de Geus EJ, Posthuma D, Ijzerman RG, Boomsma DI. Comparing blood pressure of twins and their singleton siblings: being a twin does not affect adult blood pressure. Twin Res. 2001;4:385-391

5. IJzerman RG, Stehouwer CD, Boomsma DI. Evidence for genetic factors explaining the birth weight-blood pressure relation. Analysis in twins. Hypertension. 2000;36:1008-1012

6. Pietilainen KH, Kaprio J, Rasanen M, Rissanen A, Rose RJ. Genetic and environmental influences on the tracking of body size from birth to early adulthood. Obes Res. 2002;10:875-884

7. Benirschke K. Multiple Gestation. In: Resnik R, Creasy R, Iams J, editors. *Maternal-Fetal Medicine: Principles and Practice*. Philadelphia: W. B. Saunders Company; 2004. p. 55-62.

8. Silventoinen K, Magnusson PK, Tynelius P, Kaprio J, Rasmussen F. Heritability of body size and muscle strength in young adulthood: a study of one million Swedish men. Genet Epidemiol. 2008;32:341-349

9. Mackey DA, MacKinnon JR, Brown SA et al. Twins Eye Study in Tasmania (TEST): Rationale and Methodology to Recruit and Examine Twins. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2009;12:441-454

10. Sun C, Zhu G, Wong TY et al. Quantitative genetic analysis of the retinal vascular caliber: the Australian Twins Eye Study. Hypertension. 2009;54:788-795

11. Zhu G, Hewitt AW, Ruddle JB et al. Genetic Dissection of Myopia Evidence for Linkage of Ocular Axial Length to Chromosome 5q. Ophthalmology. 2008;115: 1053-1057

12. Macgregor S, Hewitt AW, Hysi PG et al. Genome-wide association identifies ATOH7 as a major gene determining human optic disc size. Hum Mol Genet. 2010;19: 2716-2734

13. Lu Y, Dimasi DP, Hysi PG et al. Common genetic variants near the Brittle Cornea Syndrome locus ZNF469 influence the blinding disease risk factor central corneal thickness. PLoS Genet. 2010;6:e1000947

14. Hur YM, Zheng Y, Huang W, Ding X, He M. Comparisons of refractive errors between twins and singletons in Chinese school-age samples. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2009;12:86-92

15. M Wright NM. Brisbane adolesecnt twin study-outline of study methods and research projects. Australian Journal of Pyschology. 2004

16. R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org

17. Kyvic KO. Generalisability and assumptions of twin studies. In: Spector TD, Sneider H, MacGregor AJ, editors. Advances in Twin and Sib-Pair Analysis. London: Greenwich Medical Media; 2000. p. 67.

18. Posthuma D, Boomsma DI. Mx scripts library: structural equation modeling scripts for twin and family data. Behav Genet. 2005;35:499-505

19. OpenMx: Multipurpose Software for Statistical Modeling. R package version 1.01. http://openmx.psyc.virginia.edu.

20. Neale MC, Cardon LR. Methodology for genetic studies of twins and families. Dordrecht: Kluwer Academic Publishers; 1992

21. Cohen J. A Power Primer. Psychol Bull. 1992;112:155-159

22. Sanfilippo PG, Hewitt AW, Hammond CJ, Mackey DA. The Heritability of Ocular Traits. Surv Ophthalmol. 2010;55:561-583

23. Barrett JF. Delivery of the Term Twin. Best Pract Res Clin Obstet Gynaecol. 2004;18:625-630

24. Joseph KS, Allen AC, Dodds L, Vincer MJ, Armson BA. Causes and consequences of recent increases in preterm birth among twins. Obstet Gynecol. 2001;98:57-64

25. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. Twin research : the official journal of the International Society for Twin Studies. 2001;4:464-477

26. Estourgie-van Burk GF, Bartels M, Boomsma DI, Delemarre-van de Waal HA. Body size of twins compared with siblings and the general population: from birth to late adolescence. The Journal of Pediatrics. 2010;156:586-591

27. Whitaker RC, Dietz WH. Role of the prenatal environment in the development of obesity. The Journal of Pediatrics. 1998;132:768-776

28. O'Connor AR, Stephenson T, Johnson A et al. Long-term ophthalmic outcome of low birth weight children with and without retinopathy of prematurity. Pediatrics. 2002;109:12-18

29. Fledelius HC. Ophthalmic changes from age of 10 to 18 years. A longitudinal study of sequels to low birth weight. II. Visual acuity. Acta Ophthalmologica. 1981;59:64-70

30. Sebris SL, Dobson V, Hartmann EE. Assessment and prediction of visual acuity in 3- to 4-year-old children born prior to term. Hum Neurobiol. 1984;3:87-92

31. Fielder AR, Moseley MJ. Environmental light and the preterm infant. Semin Perinatol. 2000;24:291-298

32. Fledelius HC. Pre-term delivery and subsequent ocular development. A 7-10 year follow-up of children screened 1982-84 for ROP. 4) Oculometric - and other metric considerations. Acta Ophthalmol Scand. 1996;74:301-305

33. Cook A, White S, Batterbury M, Clark D. Ocular growth and refractive error development in premature infants without retinopathy of prematurity. Invest Ophthalmol Vis Sci. 2003;44:953-960

34. Fledelius HC. Inhibited growth and development as permanent features of low birth weight. A longitudinal study of eye size, height, head circumference, interpupillary distance and exophthalmometry, as measured at age of 10 and 18 years. Acta Paediatr Scand. 1982;71:645-650

35. Saw SM, Tong L, Chia KS et al. The relation between birth size and the results of refractive error and biometry measurements in children. Br J Ophthalmol. 2004;88:538-542

36. He M, Hur YM, Zhang J, Ding X, Huang W, Wang D. Shared genetic determinant of axial length, anterior chamber depth, and angle opening distance: the Guangzhou Twin Eye Study. Invest Ophthalmol Vis Sci. 2008;49:4790-4794

37. Zhang J, Hur YM, Huang W, Ding X, Feng K, He M. Shared genetic determinants of axial length and height in children: the Guangzhou twin eye study. Arch Ophthalmol. 2011;129:63-68

38. O'Connor AR, Stephenson TJ, Johnson A, Tobin MJ, Ratib S, Fielder AR. Change of refractive state and eye size in children of birth weight less than 1701 g. Br J Ophthalmol. 2006;90:456-460

39. Tomazzoli L, Renzi G, Mansoldo C. Ophthalmologic follow-up of premature twins and triplets. Eur J Ophthalmol. 2003;13:439-444

40. Carbonaro F, Andrew T, Mackey DA, Young TL, Spector TD, Hammond CJ. Repeated measures of intraocular pressure result in higher heritability and greater power in genetic linkage studies. Invest Ophthalmol Vis Sci. 2009;50:5115-5119

41. Samarawickrama C, Huynh SC, Liew G, Burlutsky G, Mitchell P. Birth weight and optic nerve head parameters. Ophthalmology. 2009;116:1112-1118

42. Sun C, Ponsonby AL, Wong TY et al. Effect of birth parameters on retinal vascular caliber: the Twins Eye Study in Tasmania. Hypertension. 2009;53:487-493

Tables

Table 1: Hypotheses of Means (M) and Variances (V) tested for all traits.

	$H0_{M,V}$		$H1_{M,V}$		$H2_{M,V}$		$H3a_{M,V}$		$H3b_{M,V}$		$H4_{M,V}$		$H5_{M,V}$	
	Tw1	Tw2	Tw1	Tw2	Tw1	Tw2	Tw1	Tw2	Tw1	Tw2	Tw1	Tw2	Tw1	Tw2
MZF	1	2	1	1	1	1	1	1	1	1	1	1	1	1
MZM	3	4	2	2	2	2	2	2	2	2	2	2	1	1
DZF	5	6	3	3	1	1	1	1	1	1	1	1	1	1
DZM	7	8	4	4	2	2	2	2	2	2	2	2	1	1
DZFM	9	10	5	6	3	4	3	4	1	2	1	2	1	1
DZMF	11	12	7	8	5	6	4	3	2	1	2	1	1	1
Sib F	13		9		7		5		3		1		1	
Sib M	14		10		8		6		4		2		1	

Hypotheses tested:

$H0_{M,V}$ – Saturated model with all parameters freely estimated.

$H1_M$ - Equal Means across twin order (only same sex twin groups).
$H2_M$ - Equal Means across same sex twin groups.
$H3a_M$ - Equal Means within twin females and twin males across opposite sex pairs.
$H3b_M$ - Equal Means within twin females and twin males across same sex and opposite sex pairs.
$H4_M$ - Equal Means across twins and siblings (same sex only).
$H5_M$ - Equal Means across females and males.

$H1_V$ - Equal Variances across twin order (only same sex twin groups).
$H2_V$ - Equal Variances across same sex twin groups.
$H3a_V$ - Equal Variances within twin females and twin males across opposite sex pairs.
$H3b_V$ - Equal Variances within twin females and twin males across same sex and opposite sex pairs.
$H4_V$ - Equal Variances across twins and siblings (same sex only).
$H5_V$ - Equal Variances across females and males.

Table 2 – Descriptive statistics of traits examined.

Trait	N (Grand MZ/DZ/Sib)	Mean (Grand MZ/DZ/Sib)	SD (Grand MZ/DZ/Sib)	Min	Max	Skew	Kurtosis	Shapiro-Wilks p
Age	2053 762/1096/195	20.64 20.67/18.90/22.48	11.78 14.17/10.46/5.03	5	90	2.02	5.61	<0.0001
Birthweight (kg)	1693 644/911/138	2.56 2.40/2.54/3.43	0.65 0.63/0.56/0.59	0.67	4.76	-0.01	0.21	<0.001
Gestation (weeks)	1819 671/1013/135	37.04 36.51/37.03/39.72	3.02 3.22/2.83/1.54	24	42	-1.17	1.35	<0.0001
Adult Height (cm)	1464 540/747/177	170.67 169.22/171.43/171.91	10.04 10.12/10.05/9.25	140	200	0.11	-0.44	<0.0001
Adult Weight (cm)	1481 546/761/174	67.95 67.14/67.75/71.39	15.30 14.74/15.08/17.44	34	172	1.14	2.78	<0.0001
IPD (mm)	1735 636/912/187	60.95 60.66/60.73/63.03	3.79 3.97/3.62/3.34	44	74	-0.25	0.84	<0.0001
VA (logMAR)	2019 750/1077/192	-0.03 -0.03/-0.03/-0.09	0.14 0.14/0.15/0.09	-0.42	1.1	2.14	10.52	<0.0001
SE (D)	2041 754/1092/195	-0.17 -0.26/-0.04/-0.52	1.47 1.42/1.48/1.48	-17.75	6.44	-2.1	16.63	<0.0001
CC (D)	1900 698/1007/195	43.97 44.11/43.9/43.8	1.49 1.52/1.46/1.47	39.44	51.75	0.19	0.52	<0.001
AL (mm)	1839 664/981/194	23.20 23.22/23.14/23.48	0.87 0.89/0.83/0.94	20.02	28.29	0.45	2.48	<0.0001
ACD (mm)	1000 404/456/140	3.61 3.62/3.62/3.69	0.34 0.35/0.94/0.28	1.79	4.5	-1.44	4.59	<0.0001
CCT (μm)	1852 664/994/194	544.02 542.68/544.22/547.57	34.73 35.12/34.27/35.58	381.5	679.5	0.21	0.75	<0.0001
IOP (mmHg)	2012 745/1072/195	15.90 16.17/15.52/16.62	3.00 2.82/3.1/2.77	6	26	0.01	-0.07	<0.001
Disc Area (mm^2)	1584 605/857/122	2.08 2.07/2.09/2.11	0.40 0.41/0.39/0.39	1.15	4.04	0.29	0.05	<0.0001
Cup Area (mm^2)	1584 605/857/122	0.45 0.48/0.43/0.48	0.29 0.30/0.30/0.25	0.01	2.1	0.14	-0.19	0.003
Rim Area (mm^2)	1584 605/857/122	1.63 1.59/1.65/1.63	0.32 0.33/0.31/0.3	0.54	3.11	0.25	0.29	<0.0001
CRAE	2016 753/1069/194	165.08 164/165.91/164.47	13.63 14.05/13.32/13.45	113.69	236.94	0.25	0.45	0.004
CRVE	2016 753/1069/194	248.64 247.39/249.63/248.06	18.85 18.8/18.61/20.08	140.26	325.7	0	0.9	<0.0001

Abbreviations:

IPD – Inter-Pupillary Distance
VA – Visual Acuity
SE – Spherical Equivalent Refractive Error
CC – Corneal Curvature
AL – Axial Length
ACD – Anterior Chamber Depth
CCT – Central Corneal Thickness
IOP – Intra-Ocular Pressure
CRAE - Central Retinal Artery Equivalent
CRVE - Central Retinal Vein Equivalent

Table 3: Likelihood ratio chi-square values (χ^2) for Hypotheses regarding Means (M) and Variances (V) of birth, anthropometric and ophthalmic traits in 929 twin pairs and 195 siblings.

		Difference Log Likelihood (LL) values																
Hypotheses	d.f.	Birthweight†	Gestation†	Adult Height	Adult Weight	IPD‡	VA	SE	CC‡	AL‡	ACD	CCT	IOP	Disc Area	Cup Area	Rim Area	CRAE‡	CRVE
Remove BW				48.72*	0.74	17.31*	1.22	0.14	67.8*	30.67*	0.04	1.52	0.87	0.34	1.00	1.40	17.94*	0.60
$H1_M$	4	9.98	1.89	5.21	4.16	9.80	10.92	11.85	18.36	5.24	11.36	2.05	1.20	2.86	2.59	2.02	2.95	10.89
$H2_M$	2	11.95	9.68	13.07	6.00	24.72*	118.21*	62.32*	17.62*	1.95	36.61*	2.90	17.10	1.73	1.82	1.55	1.53	17.11*
$H3a_M$	2	0.16	0.43	0.76	3.04	4.67	1.54	0.89	0.65	1.90	1.30	1.94	0.18	0.18	0.21	0.30	0.34	0.07
$H3b_M$	2	2.19	2.47	5.51	1.59	2.28	13.21	2.97	6.55	1.09	4.82	13.44	4.32	3.41	2.13	1.28	5.75	1.80
$H4_M$	2	147.58*	229.32*	11.23	4.10	21.15*	54.50*	5.47	12.52	20.10*	11.37	0.59	35.84*	2.70	7.78	5.77	15.47	1.67
$H5_M$	1	20.01*	4.99	153.09*	45.54*	56.30*	3.17	1.19	2.82	1.75	0.02	0.35	0.45	2.31	0.24	3.54	0.95	6.00
$H1_V$	4	1.84	3.16	4.45	9.10	3.03	24.18*	12.84	4.26	3.54	25.18*	2.39	0.18	1.08	0.75	5.71	2.51	0.40
$H2_V$	2	10.75	20.55*	1.31	3.14	2.12	2.94	5.89	1.71	6.70	3.70	0.81	5.41	0.52	1.57	2.10	1.74	0.84
$H3a_V$	2	2.67	0.38	2.51	1.64	0.04	3.28	0.47	2.05	3.47	1.21	1.01	0.11	3.92	1.00	1.39	0.61	1.19
$H3b_V$	2	0.73	0.76	1.65	0.51	4.01	4.71	2.45	0.03	3.17	0.18	3.37	0.57	2.56	0.38	4.37	2.18	1.47
$H4_V$	2	87.19*	0.30	1.07	3.53	7.27	16.43	0.38	2.21	2.26	13.21	2.75	1.45	1.88	6.98	1.57	0.56	1.35
$H5_V$	1	0.92	0.91	5.96	30.14*	4.90	4.02	46.38*	1.21	1.69	3.49	1.79	3.96	0.04	3.90	0.84	0.02	1.51

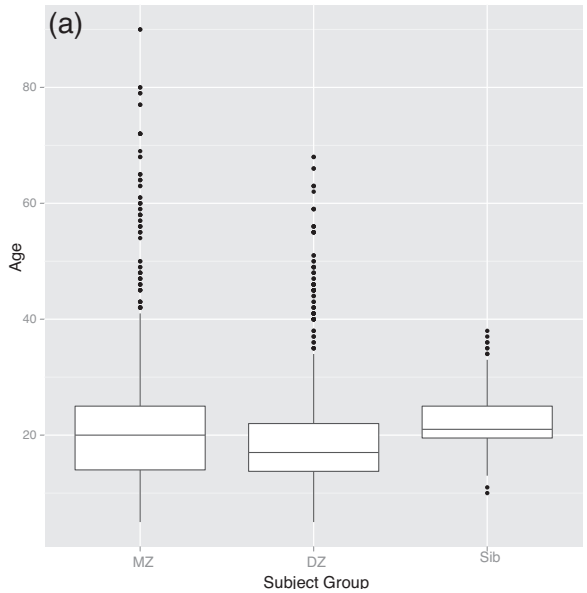
* P < 0.00023
† Unadjusted for covariate effects
All anthropometric and ophthalmic traits have been adjusted for age, age^2, sex*age, and sex*age^2.
‡ Additionally adjusted for Birthweight
The effect of Birthweight was examined by comparing the fit of the saturated model to a model with the parameter removed. If the difference in LL was significant, BW was retained as a covariate in hypothesis testing, otherwise it was eliminated.

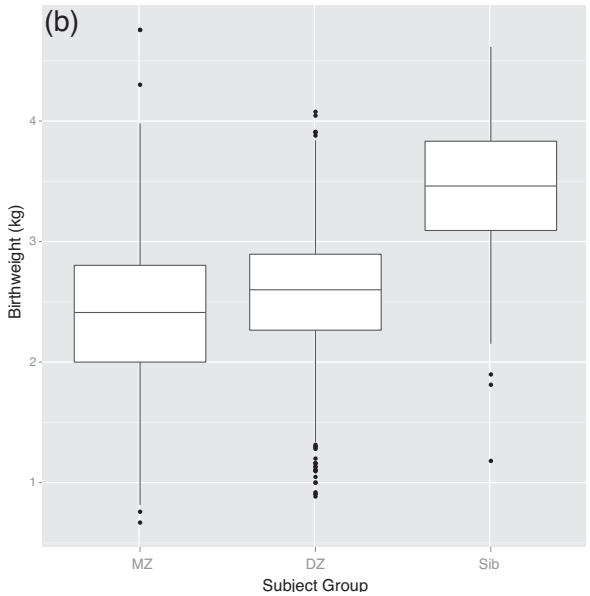
Abbreviations:

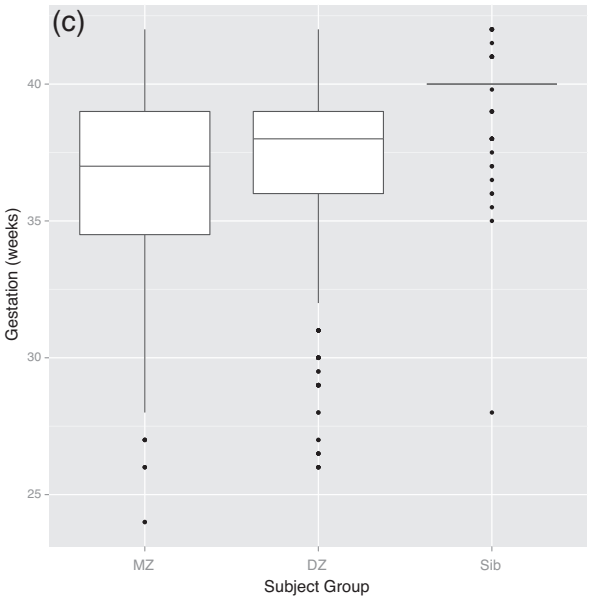
IPD – Inter-Pupillary Distance
VA – Visual Acuity
SE – Spherical Equivalent Refractive Error
CC – Corneal Curvature
AL – Axial Length
ACD – Anterior Chamber Depth
CCT – Central Corneal Thickness
IOP – Intra-Ocular Pressure
CRAE - Central Retinal Artery Equivalent
CRVE - Central Retinal Vein Equivalent

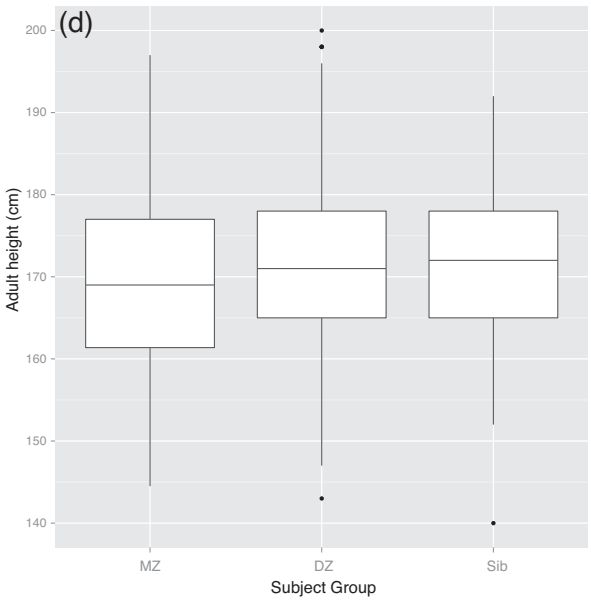
Figure Legends

Figure 1 – Boxplots for age, birth and anthropometric traits for twins (stratified by zygosity) and siblings.


(a)
Age
80
60
40
20
MZ
DZ
Sib
Subject Group


(b)
Birthweight (kg)
4
3
2
1
MZ
DZ
Sib
Subject Group


(c)
Gestation (weeks)
40
35
30
25
MZ
DZ
Sib
Subject Group


(d)
Adult height (cm)
200
190
180
170
160
150
140
MZ
DZ
Sib
Subject Group

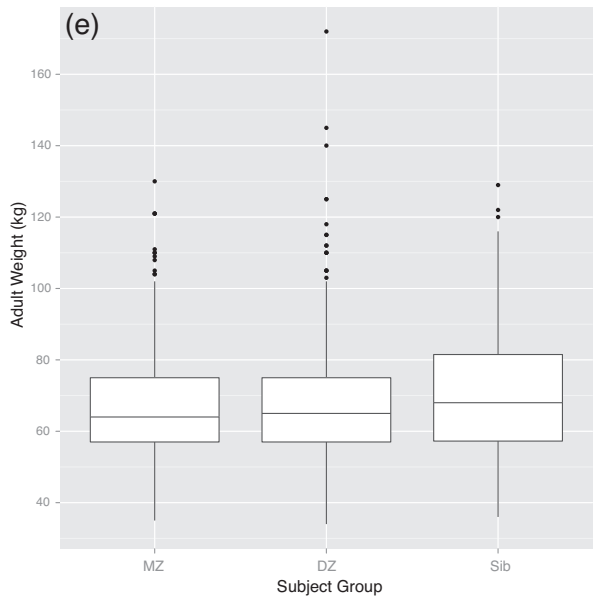
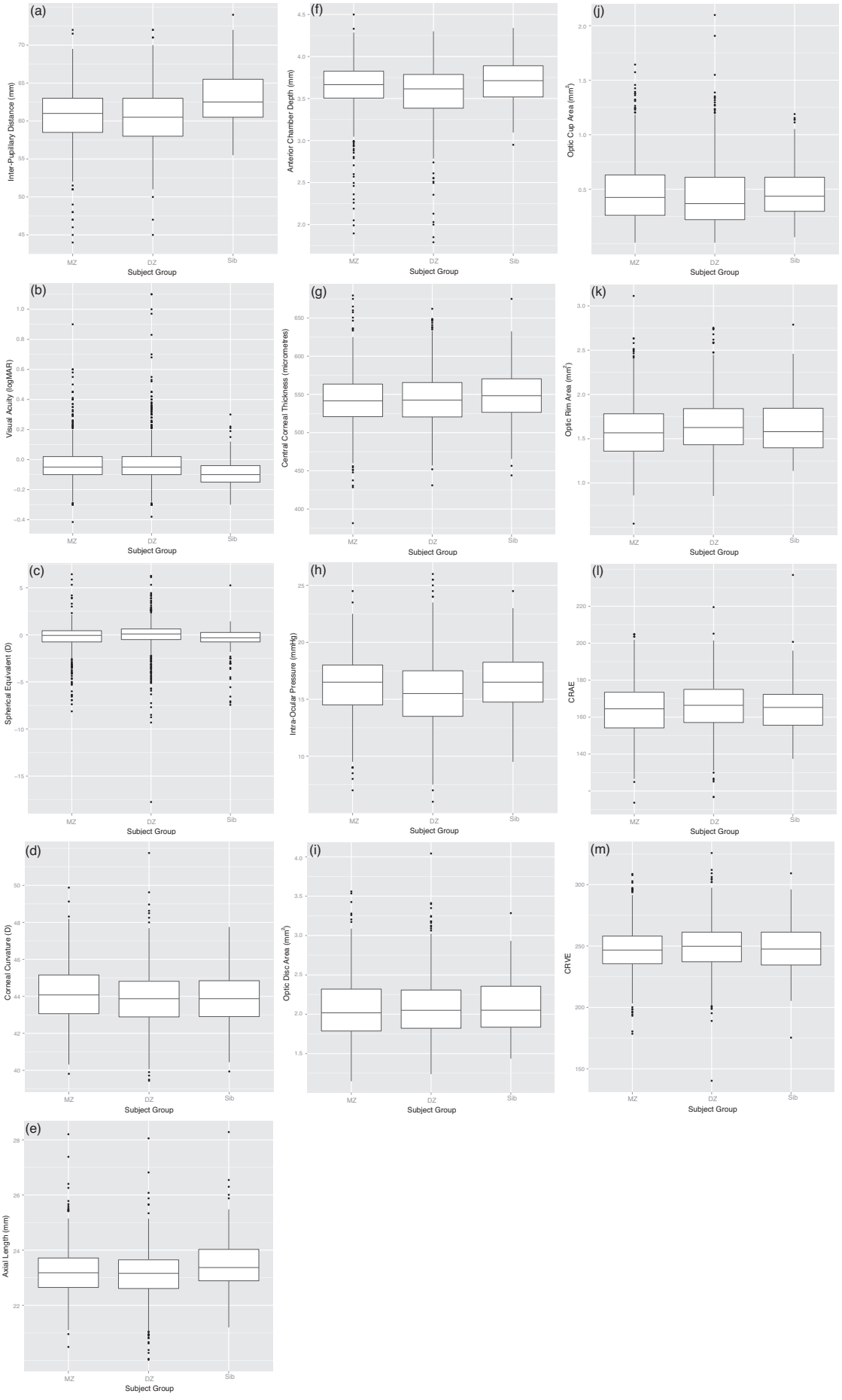

(e)
Adult Weight (kg)
160
140
120
100
80
60
40
MZ
DZ
Sib
Subject Group

Figure 2 – Boxplots for ocular biometric traits for twins (stratified by zygosity) and siblings.


(a)
Inter-Pupillary Distance (mm)
MZ
DZ
Sib
Subject Group
(b)
Visual Acuity (logMAR)
(c)
Spherical Equivalent (D)
(d)
Corneal Curvature (D)
(e)
Axial Length (mm)
(f)
Anterior Chamber Depth (mm)
(g)
Central Corneal Thickness (micrometres)
(h)
Intra-Ocular Pressure (mmHg)
(i)
Optic Disc Area (mm²)
(j)
Optic Cup Area (mm²)
(k)
Optic Rim Area (mm²)
(l)
CRAE
(m)
CRVE