*Preface: I’m choosing to write up Methods and Results for the biometric models I presented on in class (ASD\_ACE.Rmd, uploaded to GitHub). Given previous evidence in school-aged children indicating that autistic traits are highly heritable, we predicted heritability on the order of roughly 70-90% for our social communicative (SCI) and repetitive behaviors (RRB) factors. However, it is unclear (1) whether we should expect genetic effects to be this large in infancy and (2) how these genetic effects may change over the course of early development (i.e., 18 to 36 months).*

**Methods**

**Participants**

Three hundred and fourteen twins and their families participated in the Early Reciprocal Social Behavior Study (ERSB), a longitudinal study characterizing the development of quantitative autistic traits (QATs) from infancy through the toddler years. Autistic trait data related to this study have been published previously (cf. Marrus et al., 2015), but not as they pertain to developmental overlap between traits of autism and psychopathology. Of these 314 twins, 222 were retained for the duration of the study. The twins were epidemiologically ascertained from a record of all twin births that occurred in the state of Missouri between 2011 and 2013 (cf. Constantino et al., 2017). Recruitment protocol and participant characteristics are summarized in Table 1.

[INSERT TABLE 1]

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments. Specifically, the WUSM Human Research Protection Office (HRPO; 201208010) and the State of Missouri Department of Health and Senior Services Institutional Review Board (State IRB Approval #1296) approved all study procedures. Informed consent was obtained from parents of twins who participated in the study.

**Measures**

Measures were completed by the consenting individual. Descriptive statistics for all measures are reported in Table 2; information regarding their reliability and validity are reported in Marrus et al. (2015).

[INSERT TABLE 2]

**Video-referenced rating of Reciprocal Social Behavior.** A video-referenced rating of Reciprocal Social Behavior (vrRSB; Marrus et al., 2015) was used to ascertain autistic traits at baseline. It is a 44-item quantitative autistic trait scale suitable for children 12 through 24 months of age. To help parents make nuanced evaluations of variations in early social communication, the vrRSB provides a 3-minute video to serve as a ‘scoring anchor.’ In this video, a 19-month old child interacts with several adults. Throughout the video, critical early social behaviors (e.g., turn-taking, motivation to engage, responsiveness to social cues) are portrayed. The scoring anchor is designed to provide a naturalistic benchmark against which to evaluate early childhood behavior, thereby standardizing informants’ responses to items. Following the video, parents compare their child’s behavior to behavior portrayed in the video scoring anchor for 13 “video-referenced” items probing aspects of social communication and interaction. The remaining 31 “non-video-referenced” items also assess behaviors related to DSM5 clinically important domains of ASD, specifically, social communication and interaction (*SCI*) and restricted interests and repetitive behavior (*RRB*; Marrus et al., 2015). The *vrRSB Total* score quantifies reciprocal social behavior (RSB; hereafter, *vrRSB Total* will be referred to as *RSB*) and consists of *SCI* and *RRB* subscales. Higher *RSB, SCI,* and *RRB* scores indicate greater impairment. Importantly, *RSB* and *SCI* scores are continuously distributed, capturing trait variation in the general population (Marrus et al., 2015).

**Social Responsiveness Scale, second edition, Preschool Version.**The Social Responsiveness Scale, second edition (SRS-2; Constantino & Gruber, 2012) is a 65-item quantitative trait scale measuring autistic traits (QATs) from preschool through adulthood and requiring about 15-20 minutes to complete. The *SRS-2 Total* score quantifies QATs and encompasses both DSM5 criterion domains of ASD (*SCI* and *RRB*). Higher *Total* scores indicate greater impairment. Much like the vrRSB, *RSB* and *SCI* scores are continuously distributed in the general population (Constantino & Gruber, 2012).

**Goldsmith Child Zygosity Questionnaire.** The Goldsmith Child Zygosity Questionnaire (Goldsmith, 1991) is a 27-item parent-report measure developed to assess the degree of physical similarity between twins, from which determinations about zygosity can be made. Agreement with biological indicators of zygosity has been shown to exceed 93% (Price et al., 2000). In the present study, the Goldsmith Child Zygosity Questionnaire was administered over the phone to all families of same-sex twin pairs. Questionnaire-based zygosity determinations were genetically confirmed in 24 randomly-selected families; correspondence between questionnaire and genetic determinations was observed in all instances. Twins pairs were excluded from analyses (n twin pairs = 6) if zygosity could not be determined by questionnaire.

**Data Analysis**

Structural equation modeling (SEM) was used to fit biometric models estimating the relative contributions of genetic and environmental effects to autistic trait structure, from which heritability was calculated. Biometric analyses assume 100% genetic concordance between monozygotic (MZ) twins and 50% genetic concordance between dizygotic (DZ) twins. Following from these assumptions, the extent to which trait-level correlations are more similar for MZ than DZ twins can be used to estimate additive genetic effects (A), non-additive/dominant genetic effects (D), shared environmental effects (C), and non-shared environmental effects (E). Given our present interest in the heritability of autistic traits, we chose to fit biometric models evaluating additive genetic affects (A), shared environmental effects (C), and non-shared environmental effects (E); that is, an ACE model.

All analyses were conducted in R (R Studio, 2016). The following packages were used: lavaan (Rosseel, 2012), tidyverse (Wickham, 2017), and semPlot (Epskamp & Stuber, 2017). First, cross-sectional ACE models were fit to *SCI* and *RRB* data at 18- and 36-months, and heritability was estimated as the proportion of total variance () accounted for by additive genetic effects (; Figure 2). Next, to evaluate early developmental changes in genetic structure, Cholesky decomposition was implemented (cf. Lacourse et al., 2014; Figure 3). Cholesky decomposition quantifies the temporal consistency of genetic, shared environmental, and non-shared environmental sources of variance, and it is preferred to latent growth curve modeling when measurement instruments vary within-person across time. Longitudinal paths between different latent factors (i.e., additive genetic variance for twin 1 at 18 months and additive genetic variance for twin 2 at 36 months) were constrained to zero, and longitudinal paths between the same latent factors (i.e., additive genetic variance for twin 1 at 18 months and additive genetic variance for twin 1 at 36 months) of different groups (i.e., MZ, DZ) were constrained to be equal. Using results derived from these models, the heritability of autistic trait change from 18 to 36 months was estimated as the proportion of total variance at 36 months () accounted for by additive genetic effects at 36 months (; Figure 3). Inherent in Cholesky models, and control for genetic and environmental effects at 18 months.

[INSERT FIGURE 1]

[INSERT FIGURE 2]

**Results**

**Cross-sectional ACE models**

Fit statistics for cross-sectional ACE models are presented in Table 3 and parameter estimates are presented in Table 4. Notably, fit at 36 months was extremely poor, suggesting that associated parameter estimates may be unreliable. Poor fit it likely attributable to reduced sample size at 36 months relative to 18 months. Therefore, we will limit our discussion of results to biometric analyses on 18-month data. At 18 months, additive genetic and non-shared environmental influences accounted for a majority of trait variance in *RRB* and *SCI*, with limited influences from the shared environment (Table 4). More specifically, heritability estimates revealed that 66.3% and 47.4% of the variances, respectively, in *RRB* and *SCI* at 18 monthswere accounted for by genetic factors.

[INSERT TABLE 3]

[INSERT TABLE 4]

**Longitudinal Cholesky decomposition**

Fit statistics for Cholesky decomposition models are presented in Table 3 and parameter estimates are presented in Table 5. Despite acceptable fit, our models were unable to reliably estimate shared environmental influence for either *RRB* or *SCI* at 36 months (). This is not altogether surprisingly, given that shared environmental influence played a negligible role in cross-sectional models of *RRB* and *SCI*. Utilizing parameter estimates for and , the heritability of change in *RRB* from 18 to 36 months was estimated at 114% and the heritability of change in *SCI* from 18 to 36 months was estimated at 76%, indicating that genetic effects drive most of the changes in autistic trait expression during this early developmental epoch.

[INSERT TABLE 5]

**Tables**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 1.** Participant [percent of sample (%), number of individuals (ntwins)] and response [mean, standard deviation (SD), range] characteristics at 18 and 36 months | | | | |
|  | 18 months | | 36 months | |
|  | % | ntwins | % | ntwins |
| Gender |  |  |  |  |
| iiiMale | 49.36 | 155 | 50.00 | 111 |
| iiiFemale | 50.64 | 159 | 50.00 | 111 |
| Zygosity |  |  |  |  |
| iiiMonozygous | 34.4 | 108 | 23.4 | 52 |
| iiiDizygous | 60.5 | 190 | 72.1 | 160 |
| iiiSame sex dizygous | 37.6 | 118 | 44.1 | 98 |
| iiiOpposite sex dizygous | 22.9 | 72 | 27.9 | 62 |
| iiiUnknown | 5.1 | 16 | 4.5 | 10 |
| Race |  |  |  |  |
| iiiAmerican Indian/Alaska Native | 0.0 | 0 | 0.0 | 0 |
| iiiAsian | 1.3 | 4 | 1.8 | 4 |
| iiiBlack/African-American | 8.3 | 26 | 8.1 | 18 |
| iiiCaucasian | 79.0 | 248 | 80.2 | 178 |
| iiiNative Hawaiian/Other Pacific Islander | 0.0 | 0 | 0.0 | 0 |
| iiiMixed race | 10.8 | 34 | 9.9 | 22 |
| iiiUnknown/Other | 0.6 | 2 | 0.0 | 0 |
| Ethnicity |  |  |  |  |
| iiiHispanic | 7.0 | 22 | 5.4 | 12 |
| iiiNon-Hispanic | 91.1 | 286 | 92.8 | 206 |
| iiiUnknown | 1.9 | 6 | 1.8 | 4 |
|  | Mean (SD) | Range | Mean (SD) | Range |
| Age in months | 18.68 (1.01) | 17-23 | 35.69 (.61) | 35-39 |
| vrRSB (18 mo)/SRS-2 (36 mo) |  |  |  |  |
| iiiRSB (total) score | 21.75 (9.81) | 6-82 | 27.22 (17.9) | 0-147 |
| iiiSCI | 20.09 (8.1) | 4-65 | 24.00 (14.7) | 0-115 |
| iiiRRB | 1.67 (2.81) | 0-19 | 3.22 (3.86) | 0-32 |

Raw scores reported for behavioral assessments; RSB = reciprocal social behavior, SCI = social communication and interaction, RRB = restricted interests and repetitive behavior

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Mean (SD) | Range | Borderline clinical cut-off (%ile) | | Borderline clinical cut-off (score) | | # ≥ cut-off | | % ≥ cut-off |
| vrRSB |  |  |  | |  | |  | |  |
| iiiSCI | 20.1 (8.1) | 4 - 65 | 84 | | 28 | | 45 | | 14.3 |
| iiiRRB | 1.7 (2.8) | 0 - 19 | 84 | | 5 | | 30 | | 9.6 |
| iiiRSB | 21.8 (9.8) | 6 - 82 | 84 | | 31 | | 43 | | 13.7 |
| SRS |  |  |  | |  | |  | |  |
| iiiSCI | 24 (14.7) | 0 - 115 | 84 | | 58 | | 4 | | 1.8 |
| iiiRRB | 3.2(3.9) | 0 - 32 | 84 | | 10 | | 14 | | 6.3 |
| iiiRSB | 27.2 (17.9) | 0 - 147 | 84 | | 67 | | 6 | | 2.7 |
| \*Lower scores are of greater clinical concern | | | |  | |  | |

**Table 2.** Descriptive statistics (mean, standard deviation, range, borderline clinical cut-offs and associated sample characteristics) for study measures

**Table 3.** Fit statistics for cross-sectional ACE and longitudinal Cholesky models of *RRB* and *SCI*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | TLI | CFI | RMSEA | SRMR | Chi-square |
| *RRB* at 18 mo. | 1 | 1 | 0.02 | 0.08 | 3.1 |
| *RRB* at 36 mo. | -0.12 | 0 | 0.49 | 1.03 | 40.45 |
| *SCI* at 18 mo. | 0.9 | 0.85 | 0.27 | 0.21 | 18.78 |
| *SCI* at 36 mo. | 0.58 | 0.37 | 0.39 | 0.58 | 26.77 |
| *RRB* (Cholesky) | 0.98 | 0.99 | 0.06 | 0.16 | 4.95 |
| *SCI* (Cholesky) | 0.77 | 0.92 | 0.22 | 0.14 | 19.08 |

TLI = Tucker-Lewis Index; CFI = Comparative Fit Index; RMSEA = root mean square error of approximation; SRMR = standardized root mean square residual

**Table 4.** Parameter estimates for genetic and environmental components of cross-sectional ACE models, including standard errors (SE), p-values, and at 95% confidence intervals (CI; upper, lower)

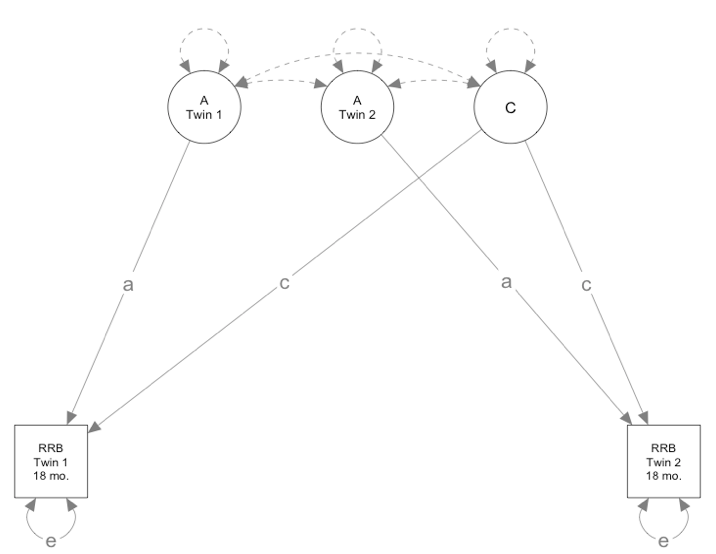
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Label | Estimate | SE | p-value | CI (upper) | CI (lower) |
| *RRB* at 18 months | a | 2.69 | 0.16 | 0 | 2.38 | 3 |
| e | 1.37 | 0.27 | 0 | 0.83 | 1.91 |
| c | 0 | 0.69 | 1 | -1.35 | 1.35 |
| *RRB* at 36 months | a | 2.77 | 0.16 | 0 | 2.44 | 3.09 |
| e | 0.5 | 0.16 | 0 | 0.19 | 0.81 |
| c | 0 | 0.46 | 1 | -0.9 | 0.9 |
| *SCI* at 18 months | a | 7.6 | 0.42 | 0 | 6.79 | 8.42 |
| e | 8.45 | 1.63 | 0 | 5.26 | 11.64 |
| c | 0 | 2.04 | 1 | -4 | 4 |
| *SCI* at 18 months | a | 6.81 | 0.39 | 0 | 6.05 | 7.58 |
| e | 2.41 | 0.73 | 0 | 0.97 | 3.85 |
| c | 0 | 1.13 | 1 | -2.22 | 2.22 |

**Table 5:** Parameter estimates for genetic and environmental components of Cholesky decomposition models at 18 months (a1, c1, e1) and 36 months (a2, c2, e2), including standard errors (SE), p-values, and at 95% confidence intervals (CI; upper, lower)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Label | Estimate | SE | p-value | CI (upper) | CI (lower) |
| *RRB* | a1 | 2.694 | 0.159 | 0 | 2.382 | 3.006 |
| c1 | -0.053 | 0.35 | 0.88 | -0.738 | 0.633 |
| e1 | 1.358 | 0.27 | 0 | 0.828 | 1.888 |
| a2 | 2.941 | 2.42 | 0.224 | -1.803 | 7.685 |
| c2 | -0.924 | NA | NA | NA | NA |
| e2 | -0.36 | 7.076 | 0.959 | -14.228 | 13.508 |
| *SCI* | a1 | 7.603 | 0.416 | 0 | 6.788 | 8.418 |
| c1 | -0.003 | 0.002 | 0.03 | -0.007 | 0 |
| e1 | 8.454 | 1.627 | 0 | 5.264 | 11.644 |
| a2 | 5.77 | 0.68 | 0 | 4.438 | 7.102 |
| c2 | 66.37 | NA | NA | NA | NA |
| e2 | 1.832 | 0.95 | 0.054 | -0.029 | 3.694 |

**Figures**

**Figure 1.** Cross-sectional ACE model depicting genetic and environmental structure of *RRB* at 18-months; to aid visualization, manifest and latent variable intercepts are not pictured. Comparable models were run for *RRB* at 36-months, *SCI* at 18-months, and *SCI* at 36-months.



**Figure 2.** Cholesky decomposition model depicting the temporal consistency of genetic, shared environmental, and non-shared environmental sources of variance for *RRB* between 18 and 36 months; to aid visualization, manifest variable intercepts, latent variable intercepts, and exogenous covariances are not pictured. A comparable model was run for *SCI*.

