**Proband autistic traits and brain development in high-risk infant siblings.**

*or*

**Familial genetic liability for autism and brain development in high-risk siblings.**

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**Word Count:**

**KEY POINTS (98 of 100 words)**

**QUESTION:** Do autistic traits in older siblings with autism spectrum disorder (ASD probands) inform brain developmental trajectories in their high-risk younger siblings?

**FINDINGS:** In this prospective longitudinal cohort study of 384 high-risk siblings, we found that proband ASD trait level was significantly associated with cortical surface area expansion and total cerebral volume growth from ages 6 to 24 months in younger siblings who received an ASD diagnosis at outcome.

**MEANING:** Proband autistic traits inform postnatal brain development in younger siblings and may serve as an important presymptomatic marker of familial genetic liability for aberrant neurodevelopment associated with ASD.

**ABSTRACT** (350 words 🡪 CURRENTLY 349)

**OBJECTIVE:** The majority of risk for autism spectrum disorder (ASD) is genetic, with younger siblings of children with ASD (probands) at increased likelihood of developing the disorder themselves. Little is known, however, about how familial genetic factors influence risk for diagnosis and brain development in high-risk siblings. This longitudinal study investigated associations between ASD traits in probands and brain development in high-risk siblings in the first two years of life.

**METHODS:** A total of 384 proband-sibling pairs were included in the study. Proband autistic traits were evaluated in relation to three primary brain phenotypes in infant siblings: total cerebral volume (TCV), total cortical surface area (TSA), and extra-axial cerebrospinal fluid (EA-CSF) derived from structural magnetic resonance images collected at 6, 12, and 24 months.

**RESULTS:** Of the 384 high-risk infant siblings (60% male), 89 (23%; 69 males [78%]) received as ASD diagnosis at the 24-month outcome visit (HR-ASD). Proband ASD trait level was associated with TCV (β = 6147.96; 95%CI 2253.39 to 1004.52; P = .002) and TSA (β = 384.94; 95%CI 107.5 to 662.37; P = .007) from 6 to 24 months of age in HR-ASD siblings. Infant siblings with probands (age range 1.7-15.5 years; 331 males [86%]) scoring above the mean for ASD traits in this cohort (n = 191; 168 male [89%]) were significantly more likely to receive an ASD diagnosis compared to those with probands scoring below the mean (73% vs 21% of HR-ASD sample; OR = 2.90, 95% CI 1.64 to 5.32; P < .001). Above-average levels of ASD traits in probands were associated with a 7.0% (Cohen’s d = 0.89, P < .001) and 5.8% (Cohen’s d = 0.64, P = .008) increase in total SA among HR-ASD siblings at 12 and 24 months of age, respectively.No associations were found between proband ASD trait level and sibling EA-CSF volumes.

**CONCLUSIONS:** These findings provide evidence that proband autistic trait level informs postnatal brain maturation in younger siblings who develop ASD. If replicated, these findings suggest that familial ASD traits may serve as important presymptomatic markers of genetic liability for aberrant neurodevelopment in ASD.

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*No more than 5 Figures/Tables*

*No more than 40 references.*

**INTRODUCTION**

Autism spectrum disorder (ASD) is a neurodevelopmental disorder diagnosed in 1 in 59 children in the United States [Baio 2018]. Mounting evidence suggests that the majority of risk for autism is genetic, with heritability estimates of 80% and above [Bia JAMA 2019; Sandin JAMA 2017]. The heritable nature of autism is reflected in recurrence rates in families, where 1 in 5 younger siblings of children with ASD will go on to receive a diagnosis themselves [Ozonoff 2011]. Prospective studies following high-risk siblings through the period of risk to first diagnosis has revealed that aberrant neurodevelopment precedes the emergence of the defining features of ASD [Girault & Piven 2020]. Despite a clarified understanding of the developmental sequalae leading up to diagnosis, the substantial phenotypic and developmental heterogeneity in ASD has hindered progress in designing effective early interventions.

A growing body of evidence suggests that there are multiple genetic pathways to ASD and that subtyping by genetic risk factors may be a tractable next step towards individualized treatments [Muhle 2018]. In the context of the high-risk sibling study design, infants who go on to develop ASD are part of multiple-incidence, or multiplex, families. Multiplex autism is more frequently associated with common polygenic variation and inherited copy number variants [Klei 2012, Leppa 2016], while simplex ASD (families with only one ASD child) is more often linked to rare de novo copy number variants and mutations [Leppa 2016]. Genetic risk scores have been generated for ASD [Grove 2020], but their predictive utility is currently limited compared to those for other neuropsychiatric disorders. One alternative way to capture polygenic risk, or genetic liability for ASD, is through the use of familial autistic traits in first-degree family members [Lyall 2014]; multiplex families have been shown to have higher recurrence risk [Ozonoff 2011, MacDonald 2020] and elevated levels of autistic traits [Schwitchenberg 2010]. This aligns with our recent report demonstrating that higher levels of autistic traits in older siblings with ASD (probands) are associated with increased risk for recurrence in their younger siblings [Girault 2020]. While it is clear that families affected by ASD harbor the greatest genetic risk, it is unknown whether *variations* in familial genetic liability for ASD contribute to the neurodevelopmental heterogeneity observed among high-risk infant siblings.

The aim of this study was to characterize associations between varying levels of genetic liability for ASD, indexed by proband autistic traits, and neurodevelopment in high-risk siblings from 6 to 24 months of age. Specifically, we examined whether autistic trait levels in probands were associated with variations in sibling neurodevelopment in three primary brain phenotypes robustly associated with autism: cerebral volume [Shen 2013, Hazlett 2011, Hazlett 2017], cortical surface area [Hazlett 2011, Hazlett 2017], and extra-axial cerebrospinal fluid volumes (EA-CSF) [Shen 2013, Shen 2017, Shen 2018]. We hypothesized that higher levels of autistic traits in probands – which confer increased risk for recurrence [Girault 2020] – would be associated with the strongest expression of autism-related brain phenotypes: larger brain volume, cortical surface area, and EA-CSF volumes. Associations were also explored for secondary brain phenotypes including regional cortical surface area [Hazlett 2017] and white matter integrity in segments of the corpus callosum [Wolff et al., 2012]. Brain phenotypes shown to be associated with autistic trait levels in probands were studied in relation to autism severity and social behaviors at 24 months in high-risk siblings.

**METHODS**

**Participants**

Younger siblings of children with ASD were recruited and enrolled as part of the Infant Brain Imaging Study (IBIS) [Estes 2015, Girault 2020]. A total of 414 HR infants were followed to outcome at 24 months. Participants were removed owing to missing phenotypic data for the proband (n = 14), the identification of a genetic syndrome or medical exclusion in the proband (n = 4), and to avoid familial bias in the dataset (n = 12) due to the enrollment of more than one younger sibling from the same family. Only one HR sibling per family was included in analyses, selected for having the most available data, and blind to diagnostic outcome. Parents provided written informed consent prior to participating in this study, and procedures were approved by the Institutional Review Boards at each clinical data collection site: University of North Carolina at Chapel Hill, University of Washington in Seattle, Children’s Hospital of Philadelphia, and Washington University in St. Louis. Further information on ascertainment and inclusion criteria are reported in the online supplemental material.

**Imaging Procedures**

Magnetic resonance imaging (MRI) data were acquired during natural sleep at 6, 12, and 24 months; MRI sample size by age are shown in TableS1 in the online supplement. T1- and T2-weighted scans (1 mm3 isotropic resolution) [Hazlett 2012, Shen 2017, Hazlett 2017] and 25-direction diffusion weighted images (DWIs) [Swanson 2015, Wolff 2017] were collected as part of the scanning protocols. Descriptions of the MRI acquisition, neuroradiological review, quality control, and cross-site reliability are detailed in previous publications on this sample [Hazlett 2012, Swanson 2015, Hazlett 2017] and in the online supplement.

**Image Analysis**

Brain volumes were obtained using a pediatric-specific, atlas-based multi-modal (T1- and T2-weighted) pipeline for probabilistic tissue classification [Hazlett 2017, Wang 2014 (Hazlett ref40)]. Total cerebral volume (TCV; reported in mm3) is defined as the summation of gray and white matter volumes of the cerebrum, including a portion of the midbrain/brainstem (see Figure S1 in the online supplement). Cortical surfaces were reconstructed using a pediatric-adapted pipeline combining both independent and within-subject longitudinal co-registrations [Hazlett 2017 & refs 41,42]. Total surface area (TSA; reported in mm2) is measured across the cerebral cortical surface, excluding the cerebellum. Extra-axial CSF (EA-CSF; reported in mm3) volumes were calculated via an automated multi-modal processing stream [Shen 2017]. EA-CSF volumes include CSF volumes in the subarachnoid space surrounding the dorsal cortical surface, excluding CSF in the ventricles and cisterns.

Secondary measures of interest included regional surface area and tract-based white matter integrity. Cortical regions shown to exhibit surface area hyper-expansion in the first year of life in infants who develop ASD [Hazlett 2017] were included in secondary analyses along with a-priori control regions (see Figure S1 in the online supplement). We also studied white matter fractional anisotropy (FA) in the genu, body, and splenium of the corpus callosum, which have been shown to follow different developmental trajectories in infants who develop ASD [Wolff 2012], or relate to ASD-relevant behaviors [Elison 2013, Swanson 2015]. A full description of the image analysis procedures can be found in the online supplement.

**Clinical Measures and Diagnostic Outcomes**

Probands were phenotyped with a battery including the Social Communication Questionnaire (SCQ) [Rutter 2003] and Autism Diagnostic Interview-Revised (ADI-R) [Rutter 2003] as described previously [Girault 2020]. The primary measure of proband ASD traits used in this study is the total score from the SCQ, where higher scored reflect the endorsement of greater numbers of autistic behaviors. At 24-month visits, HR siblings were administered a behavioral battery including the Autism Diagnostic Observation Schedule (ADOS) [Gotham 2007], Vineland Adaptive Behavior Scales (Vineland), Second Edition [Sparrow 2008], and the Communication and Symbolic Behavior Scales (CSBS) Developmental Profile [Wetherby 2002]. Sibling measures of interest for this study include ADOS calibrated severity scores [Gotham 2007], parent report of child socialization on the Vineland, and examiner-based assessment of child social skills on the CSBS. Diagnostic outcome at 24-months was determined by licensed clinicians using the DSM-IV-TR criteria. A complete description of the assessment and diagnostic procedures is reported by Estes et al. [Estes 2015].

**Statistical Analysis**

Pearson correlations were computed between proband SCQ scores and each sibling primary brain phenotype of interest (TCV, TSA, EA-CSF). These were computed separately for by diagnosis (HR-ASD, HR-NoASD) at each of the visit time points (6, 12, and 24 months). P-values and confidence intervals (CIs) were computed using a large sample approximation due to sufficient sample size [Bonnett, 2000]. P-values were adjusted using False Discovery Rate (FDR) [Benjamini, 1995] within each group (HR-ASD, HR-NoASD) and separately for primary and secondary measures of interest. Brain phenotypes shown to exhibit significant associations with proband SCQ scores were carried forward for further exploration.

Mixed effects models were employed to analyze associations between proband SCQ scores and sibling brain phenotypes longitudinally from 6 to 24 months. Each phenotype was modeled separately, with a subject-specific intercept and slope for age as random effects. Fixed effects were proband SCQ score, sibling diagnosis, sibling age, sibling sex, proband age, proband sex, and study site. Interaction terms between sibling diagnosis and proband SCQ score, and sibling diagnosis and age were also included. In secondary analyses, potential time-varying associations between proband SCQ score and sibling brain measures were modeled with a three-way interaction term (proband SCQ score x sibling diagnosis x time).

To further explore these associations, we dichotomized proband SCQ scores using a mean/median split (see Figure S2 in the online supplement), informed by evidence that proband SCQ scores in the upper quartiles conferred significantly increased risk for autism in their infant siblings [Girault et al., 2020]. Logistic regression analyses predicting sibling diagnostic outcome from proband SCQ group (above vs. below average SCQ; SCQ-High, SCQ-Low) were performed, controlling for proband and sibling age and sex, and study site. Odds ratios and CIs were computed to convey the predictive value of proband ASD trait level on recurrence risk in siblings. For brain phenotypes significantly associated with proband SCQ scores in mixed model analyses, least squares means were generated for the HR-ASD group split by proband SCQ group and plotted across time. Percent differences in model-adjusted brain phenotypes at each time point and adjusted Cohen’s *d* effect sizes were computed for HR-ASD infants based on proband SCQ group (Lipsey, 2001). Finally, correlations between proband ASD traits and primary sibling brain phenotypes were re-computed using a different but complementary measure of proband ASD traits from the ADI-R to assess the generalizability of findings beyond the SCQ.

To explore whether infant brain phenotypes explained variations in autistic and social behaviors measured at 24-month outcome, we computed Pearson correlations for the full sample of high-risk siblings. A subset of the high-risk sample (n = 80) was followed to school-age, and Pearson’s correlations between 24-month and school-age social behaviors were also computed (see online supplement for additional details). All tests were two-tailed with alpha set at 0.05. All statistical analyses were computed using the R computing software, version 3.5.1 (2018).

**RESULTS**

**Sample Description**

A total of 384 pairs of familial HR siblings (229 males; [60%]) and their probands (age range 1.7-15.5 years; 331 males [86%]) were included in the present study. At 24-month outcome, 89 HR infants (23%; 69 males [78%]) received a diagnosis of ASD (HR-ASD), the remaining 295 (77%; 160 males [54%]) did not meet criteria for ASD (HR-NoASD). **Table 1** shows sample characteristics by diagnostic group.

**Proband ASD Trait Level Explains Variation in HR-ASD Sibling Brain Phenotypes**

Correlations between proband SCQ scores and sibling brain phenotypes are depicted in **Table 2** and Figure S3 in the online supplement. Proband SCQ scores explained significant variation in TCV and TSA at 12 and 24-months in the HR-ASD, but not the HR-NoASD group. As shown in **Table 3**, linear mixed model analyses adjusting for covariates and incorporating repeated measures of brain imaging phenotypes reveal a significant proband SCQ score by sibling diagnostic group interaction for both TSA and TCV (see Table S2 in the online supplement for full model results). No significant associations were found between proband SCQ scores and EA-CSF volumes.

Regional analyses revealed that proband SCQ scores explained variation in SA measurements for HR-ASD infants in the occipital and frontal cortices, but not bilateral control regions in the premotor and parietal cortices, as shown in Figure S2and reported in Table S3 in the online supplement**.** The strongest correlations between proband SCQ scores and HR-ASD sibling regional SA were found in the right middle occipital gyrus from 6 to 24 months (0.38 ≤ r ≤ 0.44, P < 0.01), consistent with mixed model results (β= 26.76, 95% CI 10.9 to 42.61, P = .001);see Table S4 in the online supplement. Proband SCQ scores were significantly positively correlated with FA in the splenium at 6 months of age in HR-ASD infants (r = 0.45, P = 0.003), but not after. No other segments of the corpus callosum were associated with proband ASD trait level.

Potential time-varying associations between proband SCQ scores and sibling brain phenotypes were explored using a three-way interaction term (proband SCQ scores x sibling diagnostic group x time). This three-way interaction was not significant for TCV or TSA, indicating that the association between proband scores and sibling brain phenotypes were not significantly different across the interval studied. We found a marginally significant three-way interaction effect for splenium FA (-0.00014, P = .063), in line with correlation results suggesting an initial association between proband SCQ scores and sibling splenium FA at 6 months, but not later in development; model results are reported in Table S5 in the online supplement.

**HR-ASD Sibling Brain Developmental Trajectories Based on Proband ASD Trait Level**

Infant siblings with probands scoring above the sample mean for ASD traits (SCQ ≥21; n = 191, 168 male [89%]) were significantly more likely to receive an ASD diagnosis compared to those with probands scoring below the mean (73% vs 21% of HR-ASD sample; OR = 2.90, 95% CI 1.64 to 5.32; P < .001). Above-average levels of ASD traits in probands (SCQ-High) were associated with differences in sibling brain phenotypes, with a 7.0% (Cohen’s d = 0.89) and 5.8% (Cohen’s d = 0.64) increase in TSA among HR-ASD siblings at 12 months (F1,33 = 15.11, P <.001) and 24 months of age (F1,40 = 7.80, P = .008), respectively (**Figure 2b**). Similar increases were observed at 12 months (F1,33 = 4.88, P = .034) and 24 months (F1,41 = 5.15, P = .029) for TCV (**Figure 2a**). Differences were also observed in SA measures of the right middle occipital gyrus, with HR-ASD siblings of High-SCQ probands exhibiting a 18.5% (Cohen’s d = 0.96) and 12.5% (Cohen’s d = 0.63) increase in regional SA at 12 months (F1,33 = 14.89, P < .001) and 24 months (F1,40 = 7.77, P = .008), respectively (**Figure 2c**). See Table S6 in the online supplement for demographics and sample sizes for HR-ASD subjects split by proband ASD trait level.

Replacing proband SCQ scores with ADI-R reciprocal social interaction (RSI) scores yielded highly similar results, where proband ADI-R RSI scores were significantly positively correlated with TCV and TSA at 12 months (TCV: r = 0.40, P = 0.008; TSA: r = 0.47, P = 0.002) and 24 months (TCV: r = 0.33, P = 0.016; TSA: r = 0.33, P = 0.021) in the HR-ASD, but not the HR-NoASD group (see Table S7 in the online supplement).

**Sibling Brain Phenotypes and ASD Severity, Social Skills at 24-months**

Among the full sample of HR siblings, measures of TCV at 6, 12, and 24 months, and TSA at 12 and 24 months explain significant variation in 24-month ADOS symptom severity, examiner-based measures of social skills from the CSBS, and parent report of social behaviors from the Vineland (**Table 4**). For a subset of HR siblings followed longitudinally through school-age (n = 80 [64% male, 25% HR-ASD], mean age 10.5 years, range 8.0 to 11.9 years), we found significant associations between school-age Vineland socialization scores and 24-month ADOS severity scores (r = -0.32, P = .003), CSBS social composite scores (r = 0.40, P < .001), and Vineland socialization scores (r = 0.44, P <.001). This suggests brain phenotypes among HR siblings explain variation in their 24-month autistic symptoms and social skills, which in turn explain variation in school-age social behavior.

**DISCUSSION**

In this report we demonstrate that proband autistic traits, as indices of genetic liability for ASD, play an important role in both explaining risk for recurrence in multiplex families and parsing neurodevelopmental heterogeneity in infants who develop ASD. In our sample, 3 out of 4 high-risk infants who developed autism had probands with above-average levels of autistic traits. Higher levels of autistic traits in probands were also associated with the strongest expression of autism-related brain phenotypes – increased cerebral volume, cortical surface – in siblings who developed ASD. These same brain phenotypes measured in infancy were in turn related to autism severity and social behaviors in toddlerhood in the high-risk sibling cohort. Age- and phenotype-specific associations between ASD genetic liability and infant brain development across the first two years of life were also observed, in line with evidence suggesting multiple pathogenic pathways to ASD [Girault & Piven 2020]. Findings from the present study demonstrate the utility of familial autistic traits in explaining aberrant neurodevelopment in high-risk infant siblings.

In our sample, infants with the highest familial genetic liability for autism were more likely to exhibit brain overgrowth. Brain overgrowth in ASD is apparent by 12 months of age and is preceded by hyper-expansion of cortical surface area in the first year of life [Hazlett, Shen]. Our findings link variations in both cerebral volume and cortical surface area to familial genetic liability for autism across this developmental period, with ASD siblings of probands with the highest levels of autistic traits exhibiting 5-7% larger total cerebral volume and surface area at 12 and 24 months of age. Importantly, these associations were not limited to a single measure of ASD traits in probands, but were also observed with a separate measure of reciprocal social interaction. Brain-behavior analyses in high-risk siblings revealed that larger cerebral volumes and cortical surface area as early as 6 months of age was associated with greater autism severity and poorer social skills reported by parents and observed in the laboratory at 24 months. In a subset of the sample completing longitudinal follow-up, we also found that autism severity and social skills in toddlerhood were significantly associated with school-age social abilities reported more than 8 years later. Our findings align with recent reports demonstrating a link between genetic risk variants for autism and corticogenesis [Grove 2019], and between gene expression profiles in cortical neurons and autism severity in postmortem samples [Velmeschev 2019] to suggest that variations in familial genetic liability for ASD play an important role in shaping cortical development that has cascading effects on the emergence of social behaviors.

Genetic liability for autism appears to have age- and phenotype-specific associations with infant neurodevelopment. We found the most robust associations between proband ASD traits and sibling cortical development from 6 to 24 months, no associations with EA-CSF, and an association with splenium white matter integrity at 6 months, but not after. In line with findings with total surface area, regional surface area in the right middle occipital gyrus was 12-18% larger in ASD infants of probands with the highest (versus lowest) level of ASD traits, suggesting a notable role for ASD genetic liability in shaping the development of visual cortical areas, in line with evidence that visual attentional behaviors are heritable [Constantino] and disrupted prior to the ASD diagnosis [Klin, Elison, Elsabbagh]. The lack of association between ASD proband traits and sibling EA-CSF is reflective of evidence that EA-CSF is elevated regardless of familial risk [Shen 2013] and linked to motor deficits that are not specific to autism [Lorch, Nickel; see Shen review]. Thus, EA-CSF may serve as a general marker of neurodevelopmental disorders, with implications for neuroinflammatory processes [Shen review] serving as a separable component of risk for autism. Our finding that higher levels of ASD traits in probands were associated with greater white matter integrity in the splenium at 6 months of age in HR-ASD infants aligns with an earlier report in this cohort demonstrating that high-risk infants who develop autism have higher FA at 6 months of age and exhibit slower white matter growth thereafter [Wolff 2012]. This transient association with genetic liability at 6 months of age may suggest that differences in white matter integrity in the splenium in HR-ASD infants are initially driven by genetic liability for autism, but that experience-dependent mechanisms [Fields] may play a greater role in splenium development thereafter. If replicated, our findings suggest that indices of genetic liability may help to identify brain phenotypes most strongly linked to genetic risk factors (cortical volume, surface area), versus those which may arise through a separate pathophysiology (EA-CSF), or may be more modifiable by experience (white matter).

Notably, proband ASD traits were only associated with brain phenotypes in siblings who developed autism. This may reflect underlying differences in the genetic architecture of multiplex and simplex autism (i.e. inherited common variation vs. rare de-novo mutations), indicating non-shared genetic risk among sibling pairs discordant for autism. However, it is well documented that ~30% of high-risk infants who do not develop autism exhibit subthreshold features of the disorder [Ozonoff 2014, Charman. 2017], or the broad autism phenotype (BAP) [Piven 1997]. A similar pattern has been observed in neuroimaging studies, where high-risk infants without autism often represent an intermediate group between ASD infants and typically developing controls [Piven, Elison, Zylka 2017]. Thus, there is at least a subset of high-risk infants who share an inherited genetic liability for autism and express features of the BAP; additional studies will be needed to determine whether familial ASD traits may capture phenotypic variation within this BAP group.

This study has several strengths, including phenotyping of probands, and rich, prospective longitudinal neuroimaging, behavioral and clinical assessments of high-risk siblings. However, there are limitations that merit comment. Importantly, familial autistic traits are not direct measures of the complex genetic architecture of ASD, and thus can only serve as indirect indices of inherited genetic background. Future large-scale genomics studies will be needed to confirm that elevated polygenic risk for ASD is informative of recurrence risk and neurodevelopment in high-risk infants; such studies will also be necessary to yield insights into causal and contributory variants. Our findings linking proband ASD traits to sibling recurrence risk warrants replication, as two other similar studies did not find that proband ASD traits (measured via other instruments) influenced recurrence risk [Ozonoff 2012, Schwichtenberg 2011]. Limited sample sizes prevented us from exploring how genetic liability for ASD may have different effects on the male and female brain, and from linking infant brain development to school-age behavioral outcomes; the planned collection of another high-risk cohort will help to address sample size concerns in future investigations.

Findings from our study provide evidence that familial traits serve as important markers of genetic liability for ASD that inform aberrant neurodevelopment in emerging autism. Recent advances in prediction neuroscience have paved the way for identifying high-risk infants who will later be diagnosed with autism as early as 6 months of age [Emerson 2017]. The crucial next step for this work is to identify individualized areas of neurodevelopmental vulnerability [Girault & Piven 2020] that may inform targets and timing for early personalized interventions. As demonstrated in this study, and our previous report [Girault 2020], proband traits may be particularly important, cost-effective presymptomatic markers to include in such a prediction framework. Future studies of high-risk infant siblings should incorporate deep phenotyping of biological parents and siblings build a clearer picture of risk profiles at the level of the individual family and child, with the ultimate goal of maximizing the appropriateness of interventions to improve long-term outcomes.

\* Lipsey, M. W., & Wilson, D. B. (2001). Applied social research methods series; Vol. 49.Practical meta-analysis. Sage Publications, Inc.