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Gene x Environment Interactions in Developmental Dyslexia

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GENE X ENVIRONMENT INTERACTIONS IN DEVELOPMENTAL DYSLEXIA

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ABSTRACT

The goal of this project was to advance understanding of the complex multifactorial etiology of developmental dyslexia, or reading disability (RD), by investigating gene x environment (G x E) interactions. This project tested for G x E interactions using molecular genetic methods and measures of psychosocial and bioenvironmental risk factors. There are two competing predictions that can be derived from existing G x E models about the expected direction of interactions in RD. There could be "diathesis-stress" interactions in which the effects of genotype are stronger in risk environments, or there could be "bioecological" interactions in which the effects of genotype are stronger in optimal environments.

This study was a sib-pair linkage design including dizygotic twins and their non-twin siblings (age 8-19 years) from 212 families. Analyses initially focused on identifying genetic and environmental risk factors showing main effects on reading phenotypes. Sib-pair linkage analyses with two regression-based linkage models (DeFries-Fulker and Haseman-Elston) showed converging evidence for linkage in 4 regions previously associated with RD, 1p36-p34, 3p12-q13, 6p22.2, and 15q21. Across chromosomal locations, the phenotype with the strongest evidence for linkage was rapid naming. In the environmental analyses, three home variables (parental education, books in the home, and child print exposure) and two bioenvironmental variables (prenatal

exposure to smoking and birth weight) showed statistically independent main effects on child reading. The G x E analyses were conducted at the significant linkage peaks with the environments showing main effects. Both DeFries-Fulker and Haseman-Elston G x E analyses showed converging evidence for diathesis-stress G x E interaction with parent education at the chromosome 1 and 3 loci for phonological phenotypes. Follow-up analyses controlling for scaling artifacts, G-E correlations, and ADHD comorbidity revealed that the diathesis-stress G x E interactions were generally robust to these confounding factors. Discussion of the results focused on exploration of the diathesis-stress interactions in the context of previous behavioral genetic and molecular genetic findings, including dimensions that may be important for directionality of interactions, such as genetic approach (behavioral versus molecular), sample characteristics (age, disorder, and comorbidity), and environmental range.

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Chapter One

Introduction

Developmental dyslexia, or reading disability (RD), is a complex neurobehavioral disorder affecting approximately 5-10% of school-aged children (Shaywitz, Shaywitz, Fletcher, & Escobar, 1990). A consensus definition of RD was developed in 2002 by the International Dyslexia Association (IDA) and adopted by the National Institute of Child Health and Human Development (NICHD). The consensus definition emphasizes deficits in fluent word recognition that are accompanied by poor spelling and decoding abilities, all of which typically stem from underlying weaknesses in the phonological component of language. The definition also recognizes secondary consequences of RD stemming from reduced reading experience, such as reduced vocabulary growth and background knowledge, which limit a child's chances for academic and occupational success (Lyon, 1998). Moreover, there is evidence that children with reading difficulties will fall further behind their classmates over time, the so-called Matthew effect (Shaywitz et al., 2003), and so it is not surprising that RD is associated with decreases in self-esteem, motivation, and social-emotional functioning (Lyon, 1998). The academic, occupational, and psychological sequelae of RD extend the problem beyond the educational realm into the public health realm (Lyon, 1998). Although there are empirically-validated treatments available for children with RD (Shaywitz, 2003), most

children are not diagnosed until they have already fallen behind in reading and have begun to experience the negative secondary consequences of RD. Research focusing on the complex multifactorial etiology of RD, including genetic and environmental contributions and gene x environment (G x E) interactions, is needed to advance the prospects for early identification and intervention.

Knowledge of the genetic etiology of RD has been advancing at a rapid pace with the recent identification of candidate susceptibility genes for RD (for a review see Fisher & Francks, 2006; McGrath, Smith, & Pennington, 2006). Although the identification of candidate genes is a notable milestone for a complex developmental disorder like RD, these gene identifications are unlikely to answer many of the etiological questions about RD unless interactions with the environment are considered. This study will examine G x E interactions using molecular genetic methods and measures of bioenvironmental and psychosocial risk factors. This introduction will review the evidence for genetic and environmental contributions to RD, provide a rationale for the investigation of G x E interactions, and discuss predictions based on existing models of G x E interactions.

Genetic Contributions to RD

The familiality (DeFries, Fulker, & LaBuda, 1987; Hallgren, 1950) and heritability (Pennington & Olson, 2005) of RD has been firmly established. Recent heritability estimates utilizing a large twin sample showed that more than half of the variance in the group deficit could be attributed to genetic influences ($h^2_g = .58$) (Wadsworth, Olson, Pennington, & DeFries, 2000). Molecular genetic linkage studies of RD have identified and replicated several linkage peaks in the genome, a notable feat

considering that linkage findings have been notoriously difficult to replicate in complex disorders (Altmuller, Palmer, Fischer, Scherb, & Wjst, 2001). These linkage regions are often referred to as quantitative trait loci (QTLs) because they presumably contain a gene or genes that influence the quantitative trait of reading (Eley & Craig, 2005). The linkage regions have been designated by the Human Gene Nomenclature Committee (HGNC) as "DYX" as a short-hand for "dyslexia susceptibility" loci. Currently, the following seven QTLs have been replicated in linkage and/or association studies: 1p36-p34 (DYX8), 2p16-p15 (DYX3), 3p12-q13 (DYX5), 6p22.2 (DYX2), 15q21 (DYX1), 18p11.2 (DYX6), and Xq27.3 (DYX9) (for a review see Fisher & DeFries, 2002; Grigorenko, 2005a; McGrath et al., 2006; Pennington, McGrath, & Smith, in press). Two other genetic loci have been identified, 6q13-q16 (DYX4) (Petryshen et al., 2001) and 11p15 (DYX7) (Hsiung, Kaplan, Petryshen, Lu, & Field, 2004), but they await replication. This study will focus on 4 of the 7 replicated linkage regions (1p36-p34, 3p12-q13, 6p22.2, and 15q21).

Recently, six susceptibility genes in the replicated linkage regions have been proposed (for a review see Fisher & Francks, 2006; McGrath et al., 2006; Paracchini, Scerri, & Monaco, 2007). These candidate gene identifications are especially noteworthy because genes for complex behavioral disorders have proven more difficult to find than initially anticipated (Plomin, 2005). DYX1C1 (dyslexia susceptibility locus 1, candidate 1; also known as EKN1) in the 15q21 region was the first candidate gene proposed for RD (Taipale et al., 2003). There have been several attempted replications of the DYX1C1 association. Several studies found no evidence for an association (Bellini et al.,

2005; Cope, Hill et al., 2005; Marino et al., 2005; Meng, Hager et al., 2005; Scerri et al., 2004), while other studies found evidence for an association but in the opposite direction, such that the risk allele identified by Taipale et al. (2003) was the non-risk allele in these samples (Brkanac et al., 2007; Wigg et al., 2004). Only one study has replicated the association with the same causal alleles identified by Taipale et al. (2003), but this study found the strongest association with a short-term memory phenotype and weaker associations for a categorical diagnosis of RD (Marino et al., 2007). Taken together, replication evidence for DYX1C1 is mixed. Additional research is needed to disentangle whether these are false positive findings or whether the mixed findings imply genetic heterogeneity in the populations studied.

The linkage signal at 6p22 is one of the most reliably detected in RD. Two nearby candidate genes have been identified in this 6p22 region, KIAA0139 and DCDC2. Both of these genes have been replicated in independent samples. KIAA0139 has received the strongest support in UK samples (Cope, Harold et al., 2005; Francks et al., 2004; Harold et al., 2006; Paracchini et al., 2006), while DCDC2 has received the strongest support in US and German samples (Deffenbacher et al., 2004; Meng, Smith et al., 2005; Schumacher et al., 2006). As replications of each candidate gene have been completed, there has been an increasing consensus that genetic heterogeneity may explain the differing results between samples and that both candidate genes may contribute to the RD phenotype. The existence of two candidate genes in this region would provide an explanation for the reliable linkage findings across samples. Importantly, although broad

associations to both candidate genes have been replicated, none of the studies has been able to identify a replicable causal variant.

A candidate gene in the 3p12-q13 region has also been identified, ROBO1 (Hannula-Jouppi et al., 2005). This gene was identified through an individual with RD who was found to have a translocation that disrupted the ROBO1 gene. Following this discovery, the authors examined a large four generation pedigree in which the 3p12-q13 region had been implicated (Nopola-Hemmi et al., 2001). In this family, there was a SNP set in the ROBO1 region that segregated with RD (Hannula-Jouppi et al., 2005). ROBO1 has not yet been replicated as a candidate gene for RD.

The two most recent candidate gene identifications to be reported are MRPL19 and C2ORF3 on chromosome 2p12 (Anthoni et al., 2007). These genes were identified following linkage disequilibrium mapping that narrowed the candidate gene region to a small neighborhood containing 3 identified genes, FLJ13391, MRPL19, and C2ORF3. The candidate genes were further narrowed by examining brain expression and gene regulation patterns. MRPL19 and C2ORF3 are in strong linkage disequilibrium and are both highly expressed in all areas of adult brain. Interestingly, the expression of both genes correlated strongly with the 4 previously identified candidate genes (DCDC2, KIAA0319, ROBO1, DYX1C1). In contrast, FLJ13391 showed a different pattern of expression that did not correlate with the previously identified candidate genes. As a result, FLJ13391 was considered an unlikely dyslexia susceptibility candidate gene, whereas MRPL19 and/or C2ORF3 were advanced as candidate susceptibility genes (Anthoni et al., 2007). These candidate genes have not yet been replicated.

Many of the candidate genes for RD have roles in general brain developmental processes. For example, DYX1C1, KIAA0319, and DCDC2 have been implicated in neural migration (Meng, Smith et al., 2005; Paracchini et al., 2006; Rosen et al., 2007; Threlkeld et al., 2007; Wang et al., 2006) and ROBO1 has been implicated in axon guidance (Andrews et al., 2006). The cellular functions of MRPL19 and C2ORF3 are less well-characterized. Given that the genes with known functions are implicated in general brain developmental processes, future research is needed to resolve the puzzle of how disruptions in general brain development could produce a specific phenotype like RD. However, it is encouraging that the known functions of the candidate genes are consistent with landmark studies of the neuropathology of RD indicating neural migration abnormalities (Galaburda, Sherman, Rosen, Aboitiz, & Geschwind, 1985).

Overall, the identification and replication of several linkage peaks and candidate genes for RD is consistent with a multiple deficit model of RD in which several risk factors (both genetic and environmental) combine to increase susceptibility to the disorder in a probabilistic fashion (Pennington, 2006). This study will focus on 4 of the 7 replicated linkage regions (1p36-p34, 3p12-q13, 6p22.2, and 15q21) which contain 4 of the 6 candidate genes (DYX1C1, KIAA0319, DCDC2, and ROBO1).

Environmental Contributions to RD

The psychosocial environmental influences on literacy development that have received the most research attention can be grouped into 4 broad categories: home literacy environment, socioeconomic status (SES), family educational values, and home language stimulation (for a review see Phillips & Lonigan, 2005). Of course, these

variables are highly correlated and mutually influential. Each of these variables will be considered in the current study, with the exception of home language stimulation which has proven difficult to measure except through direct observation (Hart & Risley, 1992; Thorpe, Rutter, & Greenwood, 2003). I refer to these variables as "environmental" as a short-hand convenience, but I acknowledge the fact that these variables likely have genetic contributions that must be considered as well (Plomin, 1994). This issue of gene-environment (G-E) correlations will be discussed further below.

Parental education is one commonly used index of the home environment in genetic studies (Friend, DeFries, & Olson, 2008; Kremen et al., 2005). It is considered a marker variable for SES and is regarded as a "proxy for the amount of learning provided to the child, the literacy environment of the home, the parental engagement in the school, and the belief in the importance of schooling and learning" (Smith, Brooks-Gunn, & Klebanov, 1997, p. 135). Indirect evidence for this assertion comes from a study conducted by Smith et al. (1997) which focused on maternal education. This study covaried the effects of the mother's income on children's verbal ability and still found a predictive effect of maternal education. This result supports the idea that educational level contributes something above and beyond just material resources to the child's development, although it does not rule out the possibility that the relation between maternal education and child outcome is genetically mediated.

One dimension of the home literacy environment, shared reading activities between parents and children, has been the subject of considerable controversy in the literature due to debates about the magnitude of the effect (Bus, van IJzendoorn, &

Pellegrini, 1995; Dunning, Mason, & Stewart, 1994; Lonigan, 1994; Scarborough & Dobrich, 1994a, 1994b). One possible explanation for the mixed findings on the impact of shared reading on literacy development could be a G x E interaction, such that the genetic background of the sample studied may moderate the magnitude of the shared reading effect. A recent study provided preliminary evidence for such G x E interactions in language and pre-literacy development (McGrath et al., 2007).

In the current study, home literacy environment and family educational values were measured primarily through parent-report measures. Research has shown that parent-report of the home literacy environment is quite reliable and accounts for most of the variance in regressions predicting reading and language outcome, even when direct measures from home observations are included in the model (Tabors, Roach, & Snow, 2001). Nevertheless, because parent-report may be subject to social desirability influences (Stanovich & West, 1989), I also used direct measures of the child's print exposure to minimize this problem. Additionally, I used two objective measures of the home environment that are related to cognitive and language outcomes: birth order and family size (Bishop, 1997a, 1997b; Pine, 1995; Siegel, 1982; Stanton-Chapman, Chapman, Bainbridge, & Scott, 2002; Tomblin, Hardy, & Hein, 1991). These variables are thought to index the extent to which a child receives one-on-one attention from adults (Bishop, 1997b).

Although most of the research on the environmental influences impacting RD has focused on the home environment, the candidate genes for RD with known functions have all been implicated in neural development, suggesting that pre- and perinatal risk

factors may be a fruitful direction for further research. In fact, such bioenvironmental risk factors have been implicated in two disorders closely associated with RD, Attention-Deficit/Hyperactivity Disorder (ADHD) and Specific Language Impairment (SLI). Some of the identified pre- and perinatal risk factors for these two disorders are: obstetric complications (Biederman & Faraone, 2005; Bishop, 1997a; Milberger, Biederman, Faraone, Guite, & Tsuang, 1997), older maternal age at birth (Claycomb, Ryan, Miller, & Schnakenberg-Ott, 2004; Delgado, Vagi, & Scott, 2005), low birth weight and prematurity (Delgado et al., 2005; Nigg, 2006; Stanton-Chapman et al., 2002; Thapar et al., 2005), maternal prenatal smoking (Kotimaa et al., 2003; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002; Thapar et al., 2003; Tomblin, Smith, & Zhang, 1997; Wakschlag, Leventhal, Pine, Pickett, & Carter, 2006), drug/alcohol exposure (Mick et al., 2002; Nigg, 2006), and fall/winter season of birth (Seeger, Schloss, Schmidt, Ruter-Jungfleisch, & Henn, 2004). Each of these pre- and perinatal risk factors will be included in the current study.

Gene x Environment Interactions in RD

Interest in G x E interactions has flourished in recent years (e.g. Caspi & Moffitt, 2006; Grigorenko, 2005b; Kramer, 2005; Liu, Fallin, & Kao, 2004; Moffitt, 2005; Moffitt, Caspi, & Rutter, 2005; Moffitt, Caspi, & Rutter; Rutter, 2005, 2006; Rutter et al., 1997; Rutter, Moffitt, & Caspi, 2006; Rutter & Silberg, 2002; Shanahan & Hofer, 2005), leading some authors to encourage caution in the interpretation of G x E findings (e.g., Eaves, 2006). This level of activity is a notable paradigm shift in the field of behavioral and psychiatric genetics. In previous years, G x E interactions were rarely detected

(Rutter et al., 2006). Part of the difficulty with detecting G x E interactions with traditional behavioral genetic designs was that the genetic and environmental components were anonymous, but the mechanisms underlying the interaction are likely to involve specific genes and specific environments (Moffitt et al., 2005, 2006; Rutter et al., 2006). Recent studies that have tested for G x E interactions with measured genes and/or measured environments have had more success. Ideally, tests of G x E interaction would use molecular genetic methods and even specific risk alleles (e.g., Caspi et al., 2002; Caspi et al., 2005; Caspi et al., 2003). Unfortunately, in the case of RD, specific risk alleles of the candidate genes have not yet been consistently replicated across samples. Nonetheless, in the absence of identified risk alleles, molecular genetic methods for testing G x E interactions can still be implemented using adaptations of linkage models. The current project will test for G x E interactions at 4 of the 7 replicated RD linkage peaks with home environment measures and pre- and perinatal risk factors.

G x E interactions are a complex topic (Grigorenko, 2005b) and various forms of interaction are just beginning to be explored. In the recent literature, a G x E interaction typology has been advanced to categorize four different forms of G x E interaction (Shanahan & Hofer, 2005). This typology encompasses many previous models of G x E interaction (e.g., Kendler & Eaves, 1986; Rutter, 1983). Three of the four forms of interaction are relevant to the current study and will be discussed below. The typology is specific to social context environments, but the concepts can be extended to other environmental risk factors, such as biological risk factors. The first category, "Contextual Triggering," refers to the triggering of a phenotype when genetic and

environmental risk factors combine. In the psychopathology literature, contextual triggering is also referred to as the diathesis-stress model (Rende & Plomin, 1992). The diathesis-stress model predicts that the impact of genotype should be larger in risk environments (Rutter et al., 2006). The second category, "Social Context as Compensation," can be considered the opposite end of the continuum of contextual triggering, such that the lack of a risk environment will prevent the manifestation of the undesirable phenotype. The third category, "Social Context as Enhancement," refers to the ability of positive social contexts to accentuate existing genetic predispositions. This model predicts that the impact of genotype should be larger in enriched environments (Rutter et al., 2006). Two specific models fall under the umbrella of the "Social Context as Enhancement" category. First, Bronfenbrenner & Ceci (1994) advanced a "bioecological model" which predicted that enriched environments would enable underlying genetic differences between individuals to be actualized, whereas risk environments would mask the genetic differences. Secondly, Scarr (1992) proposed that environments could have a nonlinear influence across their range, such that most environments are "good-enough" to support adequate development and enable genetic differences to be actualized. Only very severe circumstances (e.g. abuse, neglect) prevent adequate development thereby suppressing individual genetic differences (Scarr, 1992).

Overall, these models can be distilled down to two competing predictions about the direction of the G x E interaction. In the case of "Contextual Triggering" and "Social Context as Compensation," heritability (in behavioral genetic studies) or heritability of the QTL (in molecular genetic studies) should increase in risk environments. For ease of

explanation, these models will be referred to as diathesis-stress G x E models, consistent with the nomenclature in the psychopathology literature. In the case of "Social Context as Enhancement," overall heritability or heritability of the QTL should increase in enriched environments (or "good-enough environments.") These models will be referred to as bioecological models, consistent with the nomenclature developed by Bronfenbrenner and Ceci (1994). Although this typology enables one to derive predictions about the direction of expected interactions, it does not offer a mechanistic explanation for G x E interactions, which is the next direction for this line of research (Rutter, 1983; Rutter & Pickles, 1991). A mechanistic explanation would need to provide information about the specific genes and specific environments involved in the interaction and the particular biological process that exists at the interface between the genes and the environments.

Although there is evidence for genetic and environmental influences on literacy development, to date, G x E interactions have been relatively neglected in reading research. In general, research investigating G x E interactions in academic and cognitive traits with measures of the home environment has tended to find bioecological G x E interactions (Friend et al., 2008; Harden, Turkheimer, & Loehlin, 2007; Harlaar, Dale, & Plomin, 2007; Kremen et al., 2005; Rowe, Jacobson, & Van den Oord, 1999; Turkheimer, Haley, Waldron, D'Onofrio, & Gottesman, 2003), although one study found evidence for diathesis-stress interactions (Asbury, Wachs, & Plomin, 2005) and one study found null effects (van den Oord & Rowe, 1998). The two behavioral genetic studies that have investigated G x E interactions in reading ability found bioecological G x E

interactions in an adult sample (Kremen et al., 2005) and in a child-adolescent sample (Friend et al., 2008). In the adult sample, the educational level of the participant's parents moderated the heritability of word recognition skills such that the heritability of word recognition in twins with highly educated parents was higher (h^2 = .69) than the heritability in twins with less-educated parents (h^2 = .21). The child-adolescent sample was drawn from the Colorado Learning Disabilities Research Center (CLDRC), which is also the sample for the current study. Consistent with the adult findings, results in this sample showed that the heritability of word recognition skills in twins with highly educated parents was higher (h^2_g = .71) than the heritability in twins with less-educated parents (h^2_g = .49).

In addition to these behavioral genetic findings of bioecological G x E interactions in RD with measures of the psychosocial environment, there is also preliminary converging evidence for bioecological G x E interactions from molecular genetic studies (McGrath et al., 2007). This study was conducted in our lab and investigated G x E interactions in a sample of children with Speech Sound Disorder (SSD). SSD is a developmental disorder characterized by delays in the production of intelligible speech (Shriberg, 2003). Children with SSD are at increased risk for RD (Gallagher, Frith, & Snowling, 2000b; Pennington & Lefly, 2001; Raitano, Pennington, Tunick, Boada, & Shriberg, 2004; Scarborough, 1990a) and so it is not too surprising that SSD has shown linkage to 4 of the 7 replicated RD linkage peaks (1p36-p34, 3p12-q13, 6p22.2, 15q21) (Smith, Pennington, Boada, & Shriberg, 2005; Stein et al., 2004). The SSD linkages on chromosome 1, 6, and 15 have been replicated in an independent sample

(Iyengar, personal communication, September 8, 2006; Miscimarra et al., 2007; Stein et al., 2006).

The study was a sib-pair linkage design in which children with SSD and their siblings were recruited (N = 60 families). The genetic analyses focused on the replicated linkage regions for RD/SSD on chromosomes 1, 6, and 15. The children were tested on phenotypic measures of speech, language, and pre-literacy skills and the parents completed questionnaires about the home language and literacy environment. G x E analyses were conducted with genomic regions and environments showing significant evidence of association with the phenotypes.

Results revealed 4 significant and trend-level G x E interactions ($p < .1$) with the chromosome 6 and 15 linkage peaks and environmental measures of maternal education, parental literacy exposure, and shared reading activities. More specifically, at the chromosome 6 locus, interactions with maternal education and parental literacy exposure predicted two pre-literacy skills, phonological awareness and rapid naming. At the chromosome 15 locus, an interaction with shared reading activities predicted vocabulary. This interaction was particularly interesting given the debate about the effect size of shared reading practices (Bus et al., 1995; Dunning et al., 1994; Lonigan, 1994; Scarborough & Dobrich, 1994a, 1994b). Interestingly, all of the interactions were in the bioecological direction, such that the heritability of the QTL was larger in enriched environments than in less optimal environments. Although the results were quite consistent and convergent with the behavioral genetic results, the results were necessarily preliminary because of the small sample size and exploratory nature of the analyses.

Nevertheless, the fact that G x E interactions were detected for pre-literacy skills at replicated RD loci suggested that further research was warranted. One aim of the current study was to follow-up these suggestive G x E results in a larger sample of children recruited for RD. Based on the pattern of findings in both behavioral genetic and molecular genetic studies, I predicted bioecological G x E interactions in RD with measures of the home environment.

In contrast to G x E research on academic and cognitive traits, research on psychopathologies has tended to find evidence for diathesis-stress interactions (e.g., Cadoret, Yates, Troughton, Woodworth, & Stewart; Caspi et al., 2002; Caspi et al., 2005; Caspi et al., 2003; Silberg, Rutter, Neale, & Eaves, 2001). The Caspi studies published in Science (Caspi et al., 2002; Caspi et al., 2003) are notable examples of diathesis-stress interactions because they were the first to identify interactions with individual risk genes. Attempted replications of these studies have been generally successful (Eley et al., 2004; Foley et al., 2004; Grabe et al., 2005; Kaufman et al., 2004; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Wilhelm et al., 2006; Zalsman et al., 2006) but there are notable exceptions (Gillespie, Whitfield, Williams, Heath, & Martin, 2005; Haberstick et al., 2005). There is also evidence for diathesis-stress interactions in ADHD, a disorder closely associated with RD. These interactions have involved measures of the pre- and perinatal environment, such as prenatal smoking (Kahn, Khoury, Nichols, & Lanphear, 2003), birth weight (Thapar et al., 2005), and season of birth (Seeger et al., 2004). Thus, although I expect to find bioecological interactions for the G x E tests in RD involving measures of the home environment, I expect to find diathesis-stress interactions with

measures of the pre- and perinatal environment based on previous findings in other developmental disorders associated with RD. An important dimension of the current project was to explore the different directions of interactions that may arise with different environmental variables in RD (e.g., bioenvironmental versus psychosocial).

Recent G x E findings in ADHD suggest that refinement of G x E models by considering different environmental variables could be theoretically very informative. For example, there have been several recent reports of diathesis-stress interactions in ADHD with measures of the psychosocial environment, such as SES and parent education (Lasky-Su et al., 2007; Laucht et al., 2007; Retz et al., 2008; Waldman, 2007). Although these interactions are consistent with previous G x E findings with pre- and perinatal risk factors in ADHD, the results are surprising given the high rates of comorbidity between RD and ADHD (25-40%) (Rucklidge & Tannock, 2002; Willcutt & Pennington, 2000). As discussed, G x E interactions in RD with psychosocial variables tend to be in the bioecological direction. So, the conflicting findings for G x E interactions with parental education in RD and ADHD point to the complexities of G x E interaction and suggest areas for further G x E model development.

Gene-Environment Correlations

One complication when studying environmental risk factors is that it is difficult to determine the extent to which a measured environment may be genetically-determined (Rutter et al., 1997; Rutter et al., 2006; Scarr & McCartney, 1983). Unlike in animal studies, environments cannot be randomly assigned in human studies and so there is always the question of whether genetic risk factors in the individual influenced their

exposure to an environment. There are several mechanisms through which environments can be responsive to genetics, termed passive, evocative, and active G-E correlations (Plomin, DeFries, & Loehlin, 1977; Scarr & McCartney, 1983). I will illustrate these three types of G-E correlations using the example of a child with RD and the environments to which he/she is exposed. Passive G-E correlation refers to the fact that parents provide family environments that are partly determined by their own genetic background. So, the child with RD may have inherited a genetic propensity for RD from a parent and this parent may not enjoy reading to their child because of their own weakness in reading, leading to even poorer reading skills in the child. Evocative G-E correlation refers to the fact that individuals evoke certain responses from others based on their genetic background. For example, the child with RD may overtly struggle with reading leading parents and teachers to suggest alternative activities or focus on other strengths of the child, thereby reducing the literacy exposure of the child. Finally, active G-E correlation refers to the fact that individuals seek out environments consistent with their own skills. So, the child with RD may avoid reading and instead seek out alternative activities, thus creating an environment with reduced literacy activities resulting in poorer reading skills in the child, even despite an adequate literacy environment in the home. In all three cases, the environments to which the child is being exposed are partly determined by the child's own genetic liabilities. There is specific evidence that these kinds of G-E correlations are operational in RD (Scarborough, Dobrich, & Hager, 1991) and language development (Gilger, Ho, Whipple, & Spitz,

2001) and such G-E correlations are likely to play a role in many domains of development.

G-E correlations create both a semantic and a statistical problem in G x E interaction research. The semantic problem is that the "environmental" variable under investigation may be partially genetically determined. In this study, most of the environmental variables I described, especially those related to the psychosocial environment, are known to be partially heritable (Plomin, 1994; Plomin, DeFries, McClearn, & McGuffin, 2008). I describe them as "environmental" as a short-hand convenience because they exist outside of the child, but I acknowledge the potential genetic contributions to these environments. Without proper statistical controls, a G x E interaction with a genetically-determined E could really be a gene x gene interaction. Although such epistatic interactions are interesting in their own right, G x E research is focused on the interface of genetics and environment.

The statistical problem is that it is difficult to disentangle the effects of G-E correlations and G x E interactions in existing genetic models, and yet both mechanisms are likely to be operating in development. In fact, G x E interactions can be falsely detected in datasets with only G-E correlations, as demonstrated in simulated datasets (Friend, personal communication, November 11, 2008; Purcell, 2002). There is preliminary evidence that statistical methods which control for the relationship between the phenotype and the environment can minimize the risk of false positive G x E interactions (Purcell, 2002). In this study, statistical controls for G-E correlations will be

employed as follow-up analyses when G x E interactions are detected to ensure that the interactions are not artifacts of the G-E correlation.

General Analytic Strategy

The overall goal of this project is to advance understanding of the multifactorial etiology of RD by examining G x E interactions using molecular genetic methods and psychosocial and bioenvironmental risk factors to predict child reading phenotypes. The approach of the study is to focus on G x E interactions with genetic and environmental factors that show main effects on the child's reading phenotype. This approach could be considered conservative in that it is statistically possible for there to be G x E interactions in the absence of main effects. However, in animal studies, where genes and environment can both be manipulated experimentally, it is quite rare to find a G x E interaction in the absence of main effects (Crabbe, Wahlsten, & Dudek, 1999; Valdar et al., 2006). Main effects of G will be investigated through linkage analyses using two different regression-based approaches. The purpose of these analyses will be to identify the most informative phenotypes and the maximum linkage signal at each of the 4 RD regions of interest (1p36-p34, 3p12-q13, 6p22.2, 15q21). Main effects of environment will be examined by screening the environmental variables for their impact on the child's reading phenotype. The G x E analyses will be conducted at the significant linkage peaks with environments that show a main effect on the phenotypes. As with the linkage analyses, two different regression-based approaches will be used to test for G x E interactions in order to assess convergence across methods. If significant G x E

interactions are detected, follow-up analyses will examine alternative explanations for the interactions, including scaling artifacts, G-E correlations, and comorbidities. The direction of any detected G x E interactions with home environmental or bioenvironmental risk factors will be of significant interest, as there is evidence for diathesis-stress and bioecological G x E interactions in the literature. Based on preliminary findings, I predict bioecological interactions with measures of the home environment and diathesis-stress interactions with measures of pre- and perinatal risk factors. Careful consideration of the directionality of G x E interactions will guide further development of G x E models in developmental disorders.

Chapter 2

Method

Participants

This study included twins and their siblings that were recruited through the Colorado Learning Disabilities Research Center (CLDRC), an ongoing study of the etiology of learning disabilities and ADHD (DeFries et al., 1997). One strength of this twin sample is that it is a population-based sample. All twin pairs between the ages of 8 and 18 years were identified, without regard to reading status, through 22 different school districts in 928 different schools in metro Denver. Parents were contacted by letter and invited to participate in the study. After initial parental consent was obtained, the twins' school records were reviewed for evidence of reading problems (e.g., low achievement test scores, referral to a tutor, reports by classroom teachers or school psychologists). If either member of the twin pair had a history of reading problems, both members of the twin pair were invited to participate in the project. A separate parallel recruitment procedure was conducted to independently identify twin pairs in which at least one of the twins exhibited ADHD symptoms. Twins who entered the study via the ADHD recruitment were included in the RD genetic analyses if they met the inclusion criteria (e.g., 1.5 SD below the comparison mean on literacy phenotypes). A comparison sample of twins without reading difficulties (or other related disorders) was also recruited.

Approximately 35% of the families who were contacted agreed to participate in the initial screening procedure, and 95% of the families in the screening sample agreed to participate in the larger study if invited.

The zygosity of same-sex twin pairs was determined using selected items from the Nichols and Bilbro (1966) questionnaire, and in ambiguous cases, was confirmed by genetic analysis. Monozygotic (MZ) twins were excluded from molecular genetic analyses. Whenever possible, biological siblings of the twin pair that were within the 8-19 age range were also tested. Additional eligibility criteria include: (1) English-speaking home, (2) Full Scale IQ score of at least 70 on the WISC-R (Wechsler, 1974) or WAIS-R (Wechsler, 1981), (3) no evidence of neurological problems, (4) no uncorrected visual or auditory deficits, and (5) no known genetic disorders or syndromes.

Molecular genetic data collection began several years after the initial twin recruitment began. As a result, only a subset of the larger twin sample had genotypic information available for analysis. The sample for this study consisted of 501 children from 212 families. Altogether, when multiple sib-ships were taken into account, there were 384 sibling pairs (dizygotic twins (DZ) and non-twin sibling pairs) available for linkage analysis. Table 1 shows individual descriptive statistics for the 501 children and family level descriptive statistics for the 212 families. The statistics in Table 1 are broken down into four groups, probands, non-identified co-twins, non-twin siblings, and comparison twins. Although the DF analyses described below will define the term "proband" with statistical cut-offs, here the term indicates children that were identified via a positive history of reading problems in their school records. Descriptives for the

non-identified co-twins (e.g., no school history of reading problems) and their non-twin siblings as well as the comparison twins are also provided. Although the comparison twins were not included in the molecular genetic analyses, the descriptives for this sample were provided because this sample was used to standardize the scores of the other groups for the genetic analyses. As one would expect based on the familiality of RD, there were several families in which both twins had a positive school record for reading problems (*N*=66). There were also some families in the sample that were recruited as part of the ADHD sample and so neither twin had a positive school history of reading problems (*N* = 33). The rest of the families (*N*=113) had 1 twin with a positive school history of reading problems. Together, these families comprise the 212 affected families in Table 1. Overall, the affected and comparison sample were representative of the demographics in the metro Denver area and were comparable to each other.

Table 1. Individual descriptives for probands, non-identified co-twins, non-twin siblings, and comparison twins and family descriptives for affected and comparison families. For these descriptives, probands are those children who were identified through school records as having reading problems. Affected families are those with at least one child identified with reading problems or ADHD symptoms through school records.

Descriptives	Proband Twins (*N*=217)	Non-identified Co-Twins (*N*=168)	Non-twin Siblings (*N*=116)	Comparison Twins (*N*=1414)
	M (*SD*) Range	*M* (*SD*) Range	*M* (*SD*) Range	*M* (*SD*) Range
Age (yrs)	10.4 (2.2) 8.0 – 18.8	10.8 (2.5) 8.0 – 18.8	12.8 (2.4) 8.0 – 19.0	11.8 (2.7) 8.0 – 19.9
Full Scale IQ	100.2 (10.5) 74 – 124	111.2 (10.8) 82 – 142	107.8 (13.9) 77 – 142	113.4 (11.3) 82 – 148
Word Recognition[1]	-2.0 (1.1) -5.4 – 1.3	-.12 (1.1) -3.5 – 2.3	-.74 (1.6) -4.5 – 2.5	.00 (1.0) -4.0 – 3.6
Gender	55.8% male	51.8% male	59.5% male	47.0% male
ADHD	33.1% ADHD	20.9% ADHD	26.2% ADHD	5.7% ADHD
		Affected Families (*N* = 212)		Comparison Families (*N* = 707)
Parent Education (yrs)[2]		14.9 (2.2) 10.5 – 20.0		15.3 (2.2) 9.0 – 21.5
Ethnicity		90.8% Caucasian		86.8% Caucasian

[1] Standardized relative to comparison sample mean and SD

[2] Mean of mother and father years of education.

Procedure

The twins and siblings completed a battery of tests, including measures of cognitive, reading, and language skills at the University of Colorado, Boulder and the University of Denver. The battery was administered over the course of two separate days with twins and their eligible siblings tested simultaneously by study personnel. The children were paid $100 for their participation. Parents completed several questionnaires, including questionnaires about their twins' birth history and the home environment. The children and their parents also gave blood samples or, alternatively, buccal samples that

underwent genetic analysis at the University of Nebraska Medical Center (UNMC). The research protocols were approved by the IRBs at the three universities.

Supplementary Data Collection. Detailed measures of the home literacy environment were not included in the initial CLDRC battery. Additionally, a measure of the twins' birth history was added to the battery several years after data collection began, so this information was missing for some of the twins. As a result, follow-up questionnaires were mailed to parents of the 212 families with genotypic data in order to gather additional information about the home literacy environment and fill-in missing data regarding the twins' birth history when necessary. The appendix includes the home literacy questionnaire that was mailed to families. Details regarding the development of this measure, which was designed for this study, are discussed below in the measures section.

IRB approval was obtained to mail questionnaires to the families and contact them via telephone to introduce the project (Jolson, 1977). Questionnaires were mailed with self-addressed, stamped envelopes. Follow-up phone calls were made once the packet of questionnaires was estimated to have arrived in the mail. The follow-up phone calls were made to introduce the project, ensure that the packet arrived, and answer any questions about participation. A second follow-up phone call was made 2-3 weeks after the initial phone call if the family responded positively but had not yet returned the questionnaires. In order to further encourage responses, parents were also given the option to fill out the questionnaires online using a secure website (surveymonkey.com).

In order to insure confidentiality, questionnaires were identified in mailings and online through family ID numbers. Parents who complete the questionnaires were reimbursed with a $10 gift card.

Of the 212 eligible families, I was able to make contact via mail or phone with 77% of the sample. Original testing dates of the families ranged from 1983 – 2007, so it is not too surprising that some of the families were unreachable. Of the families that I was able to reach, I obtained a 66% response rate, which is a 51% response rate for the entire sample. In absolute terms, I collected data from 108 families, which included 270 twins and their non-twin siblings. This response rate was an improvement over previous mailings in this sample which have ranged from 26% – 36% (Friedman, Chhabildas, Budhiraja, Willcutt, & Pennington, 2003; Tunick & Pennington, 2002).

Measures

Phenotypic Variables. The CLDRC administered measures of reading and component reading processes with significant reliability, validity, and heritability (Gayan & Olson, 2001, 2003). The phenotypic measures are listed in Table 2 below and grouped into 5 broad constructs. These constructs have been defined similarly in previous studies with this sample (e.g., Compton, Olson, DeFries, & Pennington, 2002; Gayan & Olson, 2001, 2003).

Table 2. Phenotypic Constructs.

Construct/Measure	Reliability/ Validity	Reference
Literacy		
PIAT Word Recognition	.89[a]	(Dunn & Markwardt, 1970)
Timed Oral Reading of Single Words	.93[c]	(Olson, Forsberg, Wise, & Rack, 1994)
Oral Phonological Decoding	.86[b]	(Olson et al., 1994)
Silent Phonological Decoding	.80[c]	(Olson et al., 1994)
Phonological Awareness		
Lindamood Auditory Conceptualization Test	.58 -.66[c]	(Lindamood & Lindamood, 1971)
Phoneme Deletion	.66 -.79[c]	(Olson et al., 1994)
Pig Latin Test	.58 -.79[c]	(Olson, Wise, Conners, Rack, & Fulker, 1989)
Verbal Working Memory		
Nonword Repetition	.80[b]	(Gathercole, Willis, Baddeley, & Emslie, 1994)
Digit Span Forward	.78[a]	(Wechsler, 1974)
Digit Span Backward	.78[a]	(Wechsler, 1974)
Orthographic Coding		
Orthographic Choice	.55 -.63[c]	(Olson et al., 1994)
Homonym Choice	.56 -.63[c]	(Olson et al., 1994)
PIAT Spelling	.64[a]	(Dunn & Markwardt, 1970)
Rapid Naming		
Picture Naming	.80[b]	(Denckla & Rudel, 1974; Denckla & Rudel, 1976)
Color Naming	.82[b]	(Denckla & Rudel, 1974; Denckla & Rudel, 1976)
Number Naming	.86[b]	(Denckla & Rudel, 1974; Denckla & Rudel, 1976)
Letter Naming	.86[b]	(Denckla & Rudel, 1974; Denckla & Rudel, 1976)

[a] Test-retest reliability, [b] Internal consistency, [c] Construct validity - age-adjusted correlations with other measures in the construct.

Phenotypic Data Reduction. A confirmatory factor analysis (CFA) of the phenotypic variables was performed using AMOS 16 to test the proposed factor structure

illustrated in Table 2 above. The goal of this CFA was to identify the smallest number of composites that were still theoretically meaningful because it has been suggested that composite phenotypes maximize the power of genetic analyses (Marlow et al., 2003).

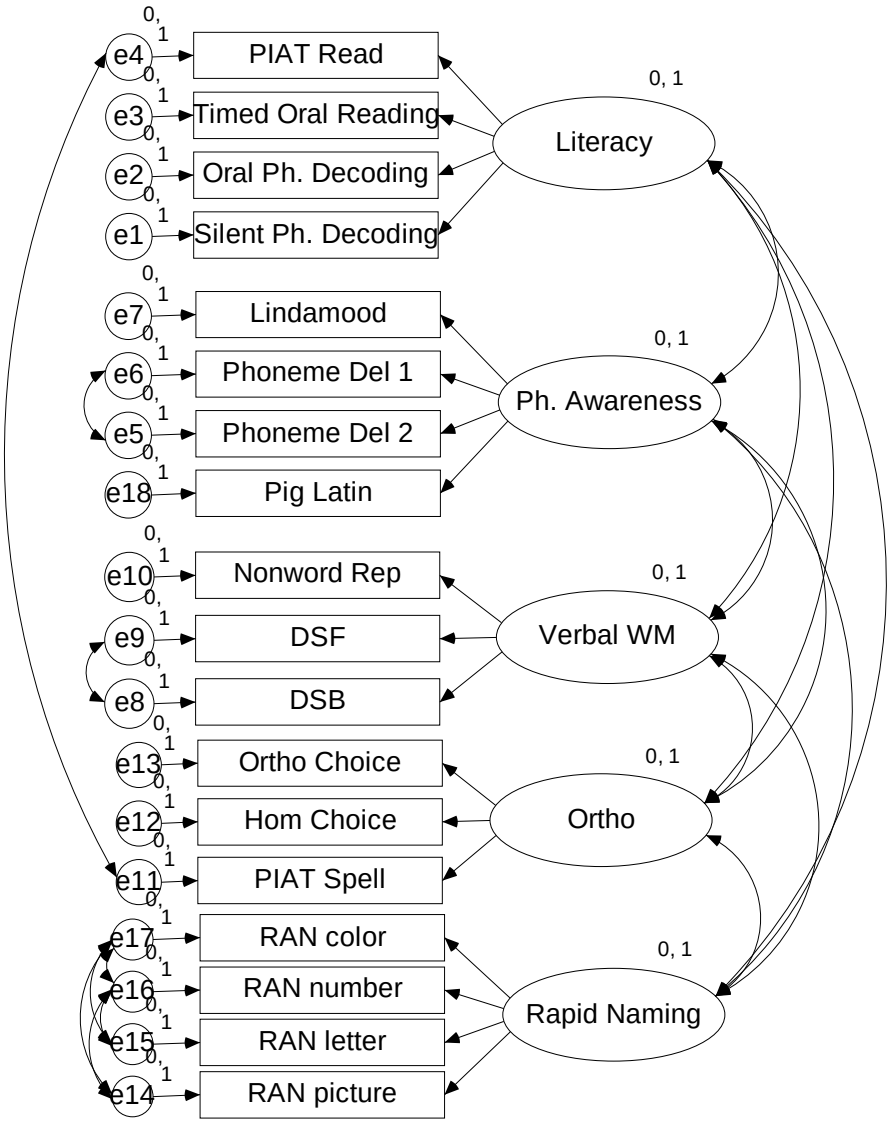
Raw scores for the phenotypic variables were age and age^2 regressed and standardized according to the comparison sample mean and SD. One child from each family (either the proband, co-twin, or sibling) was randomly selected for the CFA in order to preserve the assumption of independence. Children from affected families and comparison families were included in the analysis to maximize the generalizability of the factors. The entire sample consisted of 1,929 children.

The phenotypic variables were inspected for normality, univariate outliers, linearity, and multicollinearity. All of the variables fell below the skewness (<3) and kurtosis (<10) cut-offs recommended by Kline (2005) and so they were not transformed. Outliers that exceeded 4 standard deviations from the sample mean were winsorized to 4 standard deviations. Several scatterplots of the variables were inspected for linearity and found to be satisfactory. Correlations between the phenotypic variables loading on a single factor did not exceed the r >.85 multicollinearity cut-off recommended by Kline (2005) with the exception of the correlation between the PIAT Reading Recognition score and the Timed Oral Reading of Single Words score, r = .875. Five tasks were added to this phenotypic battery several years after the project started (Silent Phonological Decoding, Lindamood Auditory Conceptualization Test, Phoneme Deletion, Nonword Repetition, and Homonym Choice). These tasks had between 26% - 34% missing data so the data was imputed by AMOS before running the CFA. All other

variables had less than 10% missing data. I ran the CFA model with the imputed data and with listwise deletion (N=1,214 children) to insure that the imputation algorithm did not influence the results. In all cases, model fit was comparable between the imputed and listwise deleted datasets.

First, I tested the most parsimonious model, a one-factor model. This model was rejected due to poor model fit, χ^2 (127, *N*= 1929) = 2404.972, *p*<.001, χ^2/df = 18.937, Comparative Fit Index (CFI) = .892, Root Mean Square Error of Approximation (RMSEA) = .096. Next, the initial theoretical model as shown in Figure 1 below was tested. For this model and all subsequent models, the errors of subtests from the same test were allowed to correlate in order to allow for test-specific measurement error and time of testing effects (e.g., Phoneme Deletion 1 and 2, Digit Span Forward and Backward, PIAT Reading Recognition and Spelling, and the RAN tasks). One of the error correlations between the 4 RAN tasks had to be dropped because the model was not identified with all 6 correlations between the 4 tasks.

Figure 1. Original Theoretical Model



The fit of this initial theoretical model was also unsatisfactory, χ^2 (117, *N*= 1929) = 1282.761, $p<.001$, $\chi^2/df = 10.964$, CFI = .945, RMSEA = .072. I tested two alternative models that collapsed the Literacy and Phonological Awareness factor and the Literacy and Orthographic factor because these factors are closely related theoretically and they had the highest correlations in the initial model, *r* = .871, .881, respectively. The model fit significantly decreased for both of these alternative models. When I collapsed the

Literacy and Phonological Awareness factors, the following fit statistics were obtained, χ^2 (118, *N*= 1929) = 1661.841, *p*<.001, χ^2/df = 14.083, CFI = .927, RMSEA = .082, $\Delta\chi^2$ = 379.04, Δdf=1, *p*<.001. When I collapsed the Literacy and Orthographic factors, the following fit statistics were obtained, χ^2 (118, *N*= 1929) = 1549.688, *p*<.001, χ^2/df = 13.133, CFI = .932, RMSEA = .079, $\Delta\chi^2$ =266.93, Δdf=1, *p*<.001. Because neither of these alternative models provided a better fit, I considered the modification indices from our initial theoretical model. However, the suggested changes were not theoretically meaningful and so I reverted to an exploratory factor analysis (EFA) with principal axis factoring and oblimin rotation to guide the derivation of phenotypic composites. Again, this analysis was performed with 1 child randomly chosen from each family to preserve the assumption of independent observations. With listwise deletion, the resulting sample size was 1,214.

Results revealed a four factor solution according to the scree plot and an eigenvalue cut-off of 1. A loading score cut-off of .3 was set for interpretation of variables loading on a factor in the Pattern Matrix (accounts for overlapping variance between the factors) produced by SPSS. The four factors were interpretable and given the following labels: Phonological Skill, Rapid Naming, Orthographic Skill, and Verbal Working Memory. The correlations between the factors ranged from .406 - .635 so oblimin rotation was considered the best rotation choice (see Table 4). Loadings of variables on factors are presented in Table 3.

Table 3. Phenotype factor loadings resulting from Principal Axis Factoring with Oblimin rotation. Proposed labels are in italics. Loadings under .30 are not reported. Parentheses indicate cross-loadings that were not included when constructing composites.

	Factor 1 *Phonological Skill*	Factor 2 *Rapid Naming*	Factor 3 *Orthographic Skill*	Factor 4 *Verbal Working Memory*
PIAT Word Recognition	(.323)		.564	
Timed Oral Reading of Single Words			.635	
Oral Phonological Decoding	.693		(.325)	
Silent Phonological Decoding	.554		(.351)	
Lindamood Auditory Conceptualization Test	.580			
Phoneme Deletion 1	.876			
Phoneme Deletion 2	.892			
Pig Latin Test	.773			
Nonword Repetition				.374
Digit Span Forward				.705
Digit Span Backward				.476
Orthographic Choice			.727	
Homonym Choice			.826	
PIAT Spelling			.710	
RAN Color Naming		.671		
RAN Number Naming		.854		
RAN Letter Naming		.751		
RAN Picture Naming		.533		

Table 4. Factor correlation matrix

	Phonological Skill	Rapid Naming	Orthographic Skill	Verbal Working Memory
Phonological Skill	1			
Rapid Naming	.406	1		
Orthographic Skill	.635	.425	1	
Verbal Working Memory	.563	.421	.478	1

The results of this EFA were used to create four phenotypic composites. Variables that loaded >.3 on a factor were averaged together to form the composite score. Cross-loading variables were assigned to the factor on which they loaded most strongly. The composite scores were restandardized relative to the comparison sample mean and SD. Outliers were winsorized to 4 standard deviations. The resulting distributions were normally distributed (skew <|1|).

Preliminary linkage analyses with these four phenotypic composites showed that only one of the four factors, Rapid Naming, showed significant evidence of linkage (p <.01) to the replicated RD loci. None of the other factors showed even trend-level linkage signals. These results conflicted with previous linkage results with alternative phenotypes in this sample (e.g., Deffenbacher et al., 2004). Of the factors, Rapid Naming was the most homogenous factor because it was composed of 4 very similar tasks, whereas task demands of the tests composing the other factors were more varied. As a result, I constructed alternative factors with more homogeneity by examining correlations between tests within each of the originally proposed theoretical constructs. I selected the two most highly correlated variables within each factor to create the following four constructs: Word Recognition (Wrec) (Time Limited Oral Reading of Single Words and PIAT Word Recognition r = .88), Phonological Decoding (PD) (Oral Phonological Decoding and Silent Phonological Decoding, r = .83), Phonological Awareness (PA) (Phoneme Deletion and Pig Latin, r = .78), and Orthographic Coding (OC) (Orthographic Choice and Homonym Choice r = .66). These phenotypes are similar to those that have been used in previous linkage studies in this sample (Deffenbacher et al., 2004). Additionally, I continued to use the Rapid Naming (RN) factor as specified in the EFA. Table 5 below shows the correlations between the composite phenotypes in a sample with children randomly selected from each family in order to be consistent with the independence assumption (N = 1,927).

Table 5. Correlations between phenotypic composites.

	Rapid Naming (RN)	Word Recognition (Wrec)	Phonological Decoding (PD)	Phonological Awareness (PA)	Orthographic Coding (OC)
RN	1.000				
Wrec	.520**	1.000			
PD	.451**	.829**	1.000		
PA	.422**	.704**	.803**	1.000	
OC	.368**	.697**	.635**	.517**	1.000

** $p < .01$

Comorbidity

ADHD. Mothers and teachers completed a DSM-IV ADHD Rating Scale for each child similar to other DSM-IV ADHD questionnaires (Barkley & Murphy, 1998; DuPaul, Power, Anastopoulos, & Reid, 1998). Each DSM-IV ADHD symptom was rated on a four-point scale (0=not at all, 1=just a little, 2=pretty much/quite a bit, 3=very much). Analyses of ADHD comorbidity utilized continuous scores derived from this questionnaire. For these analyses, the highest rating from the child's mother or teacher was selected for each symptom, consistent with the widely utilized "Or Rule" for ADHD diagnosis (Lahey et al., 1994). Then, these symptom ratings were averaged together for the 18 symptoms included in DSM-IV.

Environmental Measures

Objective Home Environment Measures.

1.) Parent Education: Parental education is often used as a marker variable for SES (Smith et al., 1997). The CLDRC collects information about education level for both the mother and father.

2.) Family Size & Birth Order: Parents provided dates of birth for all of the children in the family which allowed the derivation of family size and birth order variables.

3.) Child Print Exposure: Children were administered four different print exposure questionnaires that were appropriate to their age: book exposure, author exposure, magazine exposure, comic exposure. These exposure questionnaires were similar to those used in other studies (e.g., Allen, Cipielewski, & Stanovich, 1992; Cipielewski & Stanovich, 1992; Cunningham & Stanovich, 1990, 1991; Echols, West, Stanovich, & Zehr, 1996; Stanovich & West, 1989; West, Stanovich, & Mitchell, 1993). The exposure questionnaires listed popular books, authors, magazines, or comic books intermingled with false lures. Children were instructed to identify the "true" items and informed that "fake" items were also included. The goal of these questionnaires was to assess children's print exposure in a format that is less susceptible to socially desirable responses than typical questionnaires (Stanovich & West, 1989). Scores for the exposure questionnaires were calculated by subtracting the number of false lures identified from the number of correctly identified items. Previous studies have shown exposure questionnaires in this format to be reliable in child samples (book exposure split-half reliability = .84, author exposure split half-reliability = .86, comic exposure split half reliability = .68) (Allen et al., 1992).

Parent- Report Home Literacy Activities - collected by the CLDRC.

These measures focused on the literacy activities of the parents with the assumption that these activities will reflect underlying values that impact the home

literacy environment for the child. However, this is an empirical question that will be tested to determine if parent literacy activities are related to child reading phenotypes.

1.) Parental Reading History Questionnaire (RHQ: Lefly & Pennington, 2000). Both parents filled out the RHQ which includes items asking them to rate on a Likert scale the degree to which they struggled with reading activities as a child (e.g., How much difficulty did you have learning to read in elementary school? 0 = none, 4 = a great deal) and the degree to which they currently participate in reading activities (e.g., How much reading do you do for pleasure? 0 = a great deal, 4 = none). The reliability and validity of the RHQ was demonstrated in a previous study (Cronbach's α = .92 - .94, test-retest reliability r = .84 - .87) (Lefly & Pennington, 2000). Subsequent analyses of the RHQ in a larger sample revealed two separate factors, current reading practices and reading history (Boada, Tunick, Raitano-Lee, Shriberg, & Pennington, under review). This measure was introduced into the study after initial data collection. As a result, many parents (45%) had missing data for this questionnaire.

2.) Parent Reading Questions: Both parents filled out three questions regarding their literacy activities. Reliability information was not available for these questions so the internal consistency of these items was tested as part of this study.

a. How many books do you read each month? None, 1-2, 3-6, 7-10, over 10

b. Estimate how many books you presently have in your home. 0-50, 51-100, 101-200, 201-500, over 500

c. Have you ever (or do you presently) read to your children? Never, Rarely, Sometimes, Regularly

Parent-Report Home Literacy Environment –supplementary mailing (see Appendix).

A home literacy questionnaire (HLQ) was developed for this study based on previous published measures with established psychometric properties (Payne, Whitehurst, & Angell, 1994; Whitehurst, Arnold, Epstein, & Angell, 1994) and measures developed in the Pennington lab and successfully implemented in G x E interaction studies (McGrath et al., 2007). The HLQ assessed the current home literacy environment as well as retrospectively assessed literacy behaviors of the parents when their twins were preschool aged. The current home literacy environment was assessed despite the variable length of time since initial testing (1983 – 2007) because there is evidence for adequate stability of the home environment based on the moderate test-retest reliability of a widely used observational measure of the global home environment, the Home Observation for Measurement of the Environment (HOME) (Bradley, 1993; Caldwell & Bradley, 1984; Totsika & Sylva, 2004). In order to empirically establish the stability of the home literacy environment in this sample, the HLQ repeated several items regarding the parent's literacy habits that were asked at the original time of testing.

The HLQ also asked parents to make retrospective judgments about shared reading activities when their participating children were in preschool. The preschool period was targeted because this period is hypothesized to be important for emergent literacy skills and the developmental trajectory of reading (DeBaryshe & Binder, 1994; Payne et al., 1994; Whitehurst et al., 1994). Because some of the twins were well into adulthood at the time of the supplementary mailing, the questionnaire directed parents to

remember events that happened during the child's preschool year as anchors for their retrospective memory (e.g. what school the child attended, what house they lived in etc.)

Questions for the HLQ were selected from previously published measures (Payne et al., 1994; Whitehurst et al., 1994) and measures developed in the Pennington lab to highlight current home literacy activities, shared reading practices during preschool, and family educational values. Additional questions asked about the estimated income of the family when the twins were in preschool (on a Likert scale) and whether the twins were enrolled in an educationally focused out-of-home care program when they were 3-4 years old. The selected questions were distributed to experts in preschool development and reading development for feedback regarding the theoretical constructs and question clarity and format. After incorporating these revisions, the questionnaire was distributed in the supplementary mailing. Further information regarding the three salient dimensions of the HLQ is provided below.

1.) Home Literacy Environment. These questions were selected from a measure developed in the Pennington lab to assess dimensions of current home literacy activities that are often neglected in existing measures, such as library visits, letter/email writing, and frequency of book purchases. The questions are given in a multiple choice format. The psychometric properties were previously examined in an exploratory factor analysis which produced a readily interpretable five factor solution: letters & library, enjoyment of books, newspaper reading, child's independent reading, and shared reading practices (McGrath et al., 2007). These results were used to guide the selection of questions for

the HLQ that would form a coherent scale indexing the richness of the literacy environment in the home.

2.) Preschool Shared Reading Practices. Questions for this dimension were selected from the Stony Brook Family Reading Survey (Payne et al., 1994; Whitehurst et al., 1994), which includes several multiple choice items designed to assess shared reading practices during the preschool period. Although the measure has been used in several studies, none of these has focused exclusively on the shared reading questions and so these specific psychometric properties were explored in the current dataset.

3.) Family Educational Values. The Stony Brook Family Reading Survey also includes several items designed to assess parental attitudes towards responsibility for the child's intellectual development. For this study, the questions most relevant to school success and reading development were selected. The items instructed the parent to mark the circle that corresponded with the balance of responsibility, for example:

Who do you think is more responsible for teaching a child new words, a teacher or a parent?

Teacher				Parent
O	O	O	O	O

The original format of the questions contained more response options, but the format was simplified to have just 5 options for the purposes of the mailing. Although Stony Brook Family Reading Survey has been used in several studies, none has focused exclusively on the educational values dimension of the scale and so these specific psychometric properties were explored in the current dataset.

In addition to these questions designed to assess educational values, two qualitative questions were included in the HLQ. These questions asked what activities parents felt families should do with young children under the age of 5 during their leisure time. I asked them to list the top 3 activities that they feel are important to do with children under 5? I asked a second question to assess whether these values were translated into activities with their children, given the time constraints that often hinder families. I asked parents to list the 3 things that they (one or both parents) did together with their twins when they were under the age of 5 years.

Pre- and Perinatal & Biological Risk Factors –collected by the CLDRC and supplementary mailing.

1.) Pregnancy and Birth Injury Questionnaire (PBIQ) from the Diagnostic Interview for Children and Adolescents – IV (DICA) (Reich, Welner, & Herjanic, 1997): Mothers were interviewed about pregnancy and birth for the twins and non-twin siblings included in the study. Questions asked information about obstetric complications, birth weight, prematurity, and prenatal exposure to smoking, drugs, and/or alcohol, including frequency of use. Because this measure was added to the battery after initial data collection began, there was missing data for some of the earlier families. Families with missing data were mailed the PBIQ along with the HLQ in the supplementary mailing.

2.) Birthweight & Prematurity: As part of the CLDRC questionnaires, parents reported birth weights for twins and siblings in the study. Parents also reported if the twins or siblings were premature and the number of weeks premature. More data was available on

this measure than on the PBIQ, so these variables were used in subsequent analyses. However, for children with information from both the CLDRC questionnaire and the PBIQ, the correlation for birth weight was r = .97 and the correlation for prematurity was r = .82. For the prematurity variable, both the continuous measure of weeks premature and a categorical designation of prematurity (<=32 weeks for twins, <=36 weeks for singletons) were used in analyses because it is possible that there is a threshold effect.

4.) Maternal Age: Mothers provided their date of birth and child's date of birth.

5.) Season of Birth: This variable was derived from the child's date of birth. The following categorical coding was used: winter =Jan, Feb, Mar; spring = April, May, June; summer = July, Aug, Sept; fall= Oct, Nov, Dec.

Environmental Data Reduction

The environmental measures were reduced to a smaller number of coherent composites. All composites were inspected for normality and outliers. Outliers were winsorized to +/- 4SD.

Of the composites that were derived below, only a subset will be selected for G x E interaction analyses based on their impact on child reading phenotypes. Before explaining the derivation of the composites, I first turn to the evidence for longitudinal stability of the home environment.

Longitudinal Stability of the Home Environment.

One assumption of the supplementary data collection was that the home literacy environment would be relatively stable. Comparisons between questions that were asked

at the initial time of testing and again in the supplementary mailing of the HLQ indicated that this was a reasonable assumption. Table 6 gives the Spearman Rho correlations between these questions.

Table 6. Spearman Rho correlations indicating longitudinal stability of the home literacy environment. (One member is randomly selected from each family for these analyses).

		Initial Data Collection				
		RHQ current attitude towards reading	RHQ current reading for pleasure	Books read per month	Books presently in the home	Have you ever (or do you presently) read to your children?
Mailing	RHQ current attitude towards reading	.488*** N=64				
	RHQ current reading for pleasure		.791*** N=64			
	Books read per month			.493*** N=89		
	Books presently in the home				.643*** N=90	
	Have you ever (or do you presently) read to your children?					.432*** N=81

**p<.001

It is important to note that aspects of the home environment that would be expected to be more stable, such as books in the home and enjoyment of reading have the highest correlations. In contrast, questions that ask about frequency of different reading activities are more likely to be influenced by more immediate family and personal circumstances.

Parent Education. Mother's and father's education were significantly correlated, r =.54 and each variable made approximately equal contributions to the child's Wrec score, standardized β = .238 for father's education, standardized β = .175 for mother's

education. As a result, mother's and father's education were averaged to create a parent education variable.

Child Print Exposure. Scores on the print exposure questionnaires were age-regressed and standardized within version against the comparison mean and SD. Table 7 shows the correlations between the variables.

Table 7. Correlations among print exposure variables (*N*=2014).

	Book Recognition	Author Recognition	Magazine Recognition	Comic Recognition
Book Recognition	1.000			
Author Recognition	.478	1.000		
Magazine Recognition	.386	.434	1.000	
Comic Recognition	.294	.339	.344	1.000

Internal consistency analysis indicated that the scale was adequate and could not be improved by deleting any of the measures (Cronbach's α =.710). As a result, scores from the 4 measures were averaged together to form a print exposure composite.

Mother and Father Home Literacy Activities & Books in Home. Parents completed three questions on the RHQ that asked about their current literacy activities as well as three literacy questions as part of the CLDRC parent battery. Correlations between mother and father ratings for these 6 questions were less than or equal to $r = 0.1$, with the exception of the parents' estimation of books in the home, $r = .695$. These low correlations between mother and father ratings indicated that separate literacy environment

composites should be created for each rater. Reliability analysis of the mother's and father's scales with all 6 items indicated that Cronbach's α could be improved by dropping the items estimating books in the home and frequency of reading to children. The resulting Cronbach's α for the mother's and father's composite were .836 and .845, respectively. Thus, scales indexing mother's and father's literacy activities were created by averaging ratings for questions about current attitudes toward reading, amount of reading for pleasure, number of books read for pleasure, and number of books read each month. An additional composite for books in the home was created by averaging mother's and father's ratings of this dimension since they were strongly correlated.

Mother's and Father's Past Reading History. In previous analyses of the RHQ, items assessing current reading practices (those described above) were less related to parental RD status than those items assessing the development of reading in childhood (Boada et al., under review). In this previous study, the authors created a scale using just the past items from the RHQ and showed good diagnostic accuracy (79%), specificity (85%) and sensitivity (70%) (Boada et al., under review). In the current study, I used the same scale derived from the past items from the RHQ in order to index the parent's genetic liability for RD. In this sample, Cronbach's α for the 9 items indexing reading history was .881 for mothers and .873 for fathers. A composite score, mother's and father's past RHQ, was created by averaging the ratings for the 9 items. The correlation between mother's and father's reading history was small but significant, $r(487) = .137$, $p < .01$. The correlation between mother's education and mother's reading history was significant,

$r(662) = .286$, $p < .001$ as was the correlation between father's education and father's reading history, $r(507) = .291$, $p < .001$. These correlations suggest the presence of G-E correlations, consistent with estimates of the heritability of psychosocial environments (Plomin et al., 2008).

Home Literacy Questionnaire.

1.) Home Literacy Environment. Inter-item correlations among the items assessing the home literacy environment on the HLQ revealed that questions assessing magazine reading and library visits were not as strongly related to the other 7 items assessing the following dimensions: parent attitude toward reading, amount of parent reading, number of books in the home, frequency of book-buying, and frequency of reading to children. When magazine reading and library visits were dropped from the scale, the resulting Cronbach's α was .76. An HLQ Home Literacy Environment composite was formed by standardizing and averaging the 7 remaining items.

2.) Preschool Shared Reading Practices. The preschool shared reading questions on the HLQ asked about the age that parents began reading to the twins, frequency of reading to the twins, frequency of library trips, and number of picture books in the home. These 4 items did not form a coherent scale (Cronbach's $\alpha = .45$). Additionally, none of these items formed a coherent scale with the HLQ home literacy environment items discussed above. Instead, inter-item correlations showed that the preschool items were fairly independent from both each other and the home literacy environment questions from the HLQ. As a result, the preschool variable indexing frequency of reading with the twins

and the variable indexing the number of picture books in the home were chosen as single indicator variables for the subsequent environmental analyses. Both of these questions captured important dimensions of the preschool literacy environment and were not significantly correlated, $r(108) = .113$, *ns.* The variable assessing age of onset of reading was not chosen as a single indicator because it was quite skewed toward the lower end, with 62% of the sample reporting that they began reading to the twins between 0-6 months, indicating that early reading was a near universal activity in this sample. Importantly, however, frequency of preschool reading showed a more normal distribution of scores.

3.) Family Educational Values. Seven questions on the HLQ assessed the family's sense of the child's, parents' and teachers' responsibility in the child's word learning, reading, and success in school. I conducted a reliability analysis using Cronbach's α to explore the properties of these items. Cronbach's α for all seven items was unsatisfactory. Instead, the pattern of correlations suggested that there were three separate factors, child's responsibility (Cronbach's $\alpha = .72$), parent's responsibility (Cronbach's $\alpha = .65$), and teacher's responsibility (Cronbach's $\alpha = .59$). Although the factors contained overlapping items (e.g. the question attributing responsibility for word learning to a teacher or a parent was include in both the teacher and parent scales), the factors were constructed to emphasize the role of the child, parent, or teacher. The composites were formed so that high scores represented larger responsibility for the skill. The correlations between the three composites are shown below in Table 8. The opposing nature of the question design means that negative correlations are expected.

Table 8. Correlations between responsibility composites (*N*=214)

	Child Responsibility	Teacher Responsibility	Parent Responsibility
Child Responsibility	1		
Teacher Responsibility	-.397***	1	
Parent Responsibility	-.349***	-.693***	1

***p<.001

4.) Out-of-Home Care. In the HLQ, I asked whether the twins attended out-of-home care when they were 3-4 years old. Eight-six percent of the sample reported that the twins attended out-of-home care, typically preschool, and 82% endorsed that the care was educationally focused. I used the categorical variable of out-of-home care in our subsequent environmental analyses.

Pregnancy and Birth Injury Questionnaire (PBIQ)

Regarding the PBIQ, it was first important to consider method variance in the administration of this measure. Typically in the CLDRC, this measure is administered via direct interview with the mother. In order to obtain missing data, this study mailed the PBIQ to families. Before merging the PBIQ data from the supplementary mailing with the existing interview data, I examined whether the two administration methods were comparable.

We considered the possibility that parents may have showed different rates of endorsement in these two administration situations. To explore this hypothesis, I examined rates of smoking and drinking in the mailing versus interview samples. Rates of smoking did not differ in the two situations, χ^2 (1, *N*= 1289) <1, *ns.* Rates of drinking

did differ between the two samples with significantly higher rates reported in the mailing sample (26.3%) than the interview sample (18.8%), χ^2 (1, *N*= 1280) = 7.442, *p* <.01. However, this effect was at least partially attributable to a cohort effect. Mothers of children born in the 1970s endorsed drinking much more frequently (44.9%) than mothers of children born in the 1980s or 1990s (18.9%, 19.7% respectively), χ^2 (2, *N*= 1272) = 18.861, *p* <.001. This cohort effect may represent a shift in medical practice and awareness. In fact, several mothers in the older cohort noted on their forms that their doctors had recommended a glass of wine during pregnancy. Because the PBIQ was added to our battery more recently, this cohort effect is confounded with the ascertainment method. Families who received the mailing tended to be families that belonged to the older cohorts. In fact, 100% of the data on children born in the 1970s was obtained by mailing; 49.1% of the data on children born in the 1980's was obtained by mailing; and only 4.5% of the data on children born in the 1990's was obtained by mailing. These percentages suggest that the differential endorsement of drinking in the mailing and interview samples is at least partially attributable to the strong cohort effect. Overall, these results suggest that data from both the mailing and interview samples can reasonably be combined without major concern about the validity of the mother's reporting in the mailing sample.

1.) Prenatal Smoking, Drinking and Drug Use. Information about prenatal smoking, drinking, and drug use was derived from the PBIQ. These variables were categorically coded (ever, never) because there were not enough endorsements in this sample to consider the impact of frequency.

2.) Obstetric Complications. An optimality index was created from the 22 variables of the PBIQ. The logic of this index is that the accumulation of minor negative events may create a risky developmental environment (Rutter, Thorpe, Greenwood, Northstone, & Golding, 2003). As a result, the prenatal smoking, drinking, and drug use items previously described were included in this measure as part of the accumulated risk events. Optimality indices have been derived from other pregnancy and birth risk measures that have overlapping items with the PBIQ (Rutter et al., 2003).

Genotyping & Ibd Estimation

Ten cc's of blood or, alternatively, buccal cell samples were obtained from twins and their siblings participating in the study and their biological parents. The PUREGENE DNA Isolation Kit (Gentra Systems) was used with minor modifications to the protocol to extract the DNA from the samples. Immediately following extraction, the preamplification extension procedure GenomiPhi (Amersham Biosciences) was used to amplify the amount of DNA. Microsatellite markers in four of the replicated RD linkage regions (1p36-p34, 3p12-q13, 6p22.2, 15q21) were selected to cover the regions with a density of approximately 2 cM. The markers and their positions are shown in Table 9. The markers were selected from the deCODE genetic map (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606). Following PCR amplification with dye-labeled primers (IDT, Coralville, IA), the ABI 3730 DNA Analyzer was used to perform genotyping. Genotypes were called with GeneMapper software (Applied Biosystems) with manual confirmation. The Genetic Analysis System (GAS) version 2 software (Young, 1995) and the MERLIN version 1.1.2 pedstats and

error features (Abecasis, Cherny, Cookson, & Cardon, 2002; Wigginton & Abecasis, 2005) were used to check for genotype errors, errors in map placement, and inheritance problems. When errors were detected by either program, the allele calls were double-checked by the technicians. The Graphical Relationship Representation (GRR: http://www.sph.umich.edu/csg/abecasis/GRR) was used to visually inspect the genetic data from the parents, twins, and siblings to insure that the biological relationships were specified correctly. Heterozygosity of these markers was calculated from the study population using the pedstats feature in Merlin 1.1.2 (Wigginton & Abecasis, 2005).

Table 9. Microsatellite markers for RD regions of interest, map positions, and heterozygosity

	Marker	Map position (cM)	Heterozygosity (observed)
Chromosome 1p36-p34	D1S2667	19.88	83.80%
	D1S2740	21.07	59.70%
	D1S507	26.24	85.70%
	D1S2672	27.22	75.80%
	D1S2697	29.37	71.40%
	D1S1592	32.19	61.50%
	D1S2826	33	65.80%
	D1S2644	35.56	78.60%
	D1S199	37.48	84.60%
	D1S478	40	76.80%
	D1S2698	42.77	73.80%
	D1S2885	44.89	87.00%
	D1S2749	46.32	76.80%
	D1S470	48.36	74.20%
	D1S2783	54.34	67.40%
Chromosome 3p12-q13	D3S1566	94.76	82.20%
	D3S3568	96.45	69.40%
	D3S3551	96.78	88.10%
	D3S3614	99.13	76.70%
	D3S3581	102.75	64.40%
	D3S3653	104.16	66.10%
	D3S3507	106.88	67.90%
	D3S3049	107.18	63.50%
	D3S1604	107.43	55.10%
	D3S1595	109.09	82.20%
	D3S1552	109.95	57.90%
	D3S1603	111.82	74.70%
	D3S3655	113.06	78.90%
	D3S1591	115.17	77.70%
	D3S3045	117.29	81.90%
	D3S1572	119.99	70.80%
	D3S3683	121.11	68.70%
	D3S1575	123.9	59.30%

	Marker	Map position (cM)	Heterozygosity (observed)
Chromosome 6p22.2	D6S1597	43.93	56.00%
	D6S1663	46.06	68.10%
	D6S461	47.05	73.80%
	D6S1554	48.86	65.90%
	D6S306	50.89	66.00%
	D6S291	55.51	70.00%
	D6S2427	58.62	76.80%
	D6S1549	61.87	56.30%
Chromosome 15q21	D15S1012	38.12	74.50%
	D15S1044	41.7	66.10%
	D15S146	41.73	68.00%
	D15S132	45.495	76.00%
	D15S143	46.31	63.30%
	D15S1028	47.82	81.20%
	D15S119	48.6	70.60%
	D15S982	50.14	72.50%
	D15S1016	51.18	92.70%
	D15S1049	53.34	75.70%
	D15S1033	56.6	73.60%
	D15S155	60.26	67.60%

Multipoint ibd estimations ($\hat{\pi}$) were calculated using the ibd feature of Merlin 1.1.2 (Abecasis et al., 2002). This software outputs the probability that the sibling pair shares 0 alleles P(0), 1 allele P(1), or 2 alleles at each marker P(2), taking into account the parental genotype information. Ibd estimations ($\hat{\pi}$) were derived from these probabilities using the following equation:

$$\hat{\pi} = (P(0) \times 0) + (P(1) \times .5) + (P(2) \times 1)$$

Linkage Approaches

The extremity-selected sib-pair design of this study requires careful consideration when choosing QTL linkage approaches. In the following, I describe the rationale behind our choice of linkage approaches and discuss the logic of the two chosen approaches. Although variance components models are among the most powerful (Feingold, 2001, 2002) and have been adapted for tests of G x E interaction (Purcell & Sham, 2002), they are not robust to violations of normality due to selected sampling (Feingold, 2001, 2002).

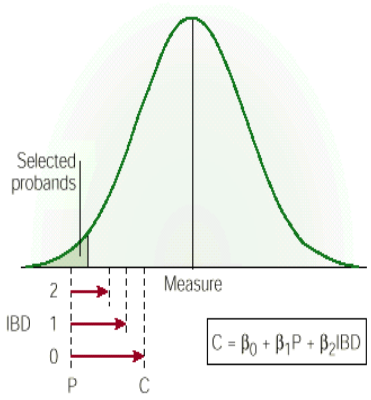
Other modeling approaches, such as Markov Chain Monte Carlo (MCMC) methods, have been adapted for tests of G x E interaction (Eaves & Erkanli, 2003), but these methods perform better with larger sample sizes and larger pedigrees than the current sample (Wijsman, personal communication, October 10, 2006). Hence, regression-based approaches are most appropriate for this sample.

The major regression-based approaches can be grouped into three broad categories: (1) Haseman-Elston (HE) and its extensions (e.g., Drigalenko, 1998; Elston, Buxbaum, Jacobs, & Olson, 2000; Forrest, 2001; Haseman & Elston, 1972; Sham & Purcell, 2001; Visscher & Hopper, 2001; Wright, 1997; Xu, Weiss, Xu, & Wei, 2000), (2) Merlin-regress (Sham, Purcell, Cherny, & Abecasis, 2002), and (3) DeFries-Fulker (DF) linkage (Fulker & Cardon, 1994; Fulker et al., 1991), which is an extension of the DF behavioral genetic analysis (DeFries & Fulker, 1985, 1988). Haseman-Elston methods are well-characterized (Feingold, 2001, 2002) and have been adapted for testing G x E interactions (Gauderman, Morrison, & Siegmund, 2001; Schaid, Olson, Gauderman, & Elston, 2003), so they are appropriate for this sample. I would also like to conduct the G x E analyses using another method to test the robustness of the results across methods. Merlin-regress was designed for use with selected samples and is robust to non-normality, but it has not yet been adapted for testing G x E interactions (Abecasis, personal communication, October 20, 2006). The DF method was developed specifically for use with highly selected probands and their siblings and has been shown to be more powerful than the original HE method when applied to selected samples (Fulker et al., 1991). The DF equation has also been adapted for tests of G x E interaction (Fulker et

al., 1991). Thus, this study utilized DF and HE linkage methods and their extensions for testing G x E interactions. These two methods have shown good correspondence in previous studies (e.g., Deffenbacher et al., 2004; Kaplan et al., 2002). DF linkage methods will be considered the primary analysis because these methods are the most powerful in selected samples (Lessem, Cherny, Abecasis, Sham, & Purcell, 2001) and can model both pair-specific and person-specific environmental variables. In contrast, HE methods are less powerful in selected samples and can only model pair-specific environmental variables.

The DF method capitalizes on the phenomenon of regression to the mean (DeFries & Fulker, 1985, 1988; Fulker & Cardon, 1994; Fulker et al., 1991). Originally, this method was developed as a behavioral genetic analysis (DeFries & Fulker, 1985, 1988) and was later extended to sib-pair linkage analyses (Fulker et al., 1991). In the DF method, at least one member of each sib-pair (the proband) is selected to be extreme on a phenotype. The logic is that, given a risk locus that affects a phenotype, a co-sib who carries the same alleles as the proband will not regress as far to the population mean as a co-sib who does not share the same genotype. In other words, if the ibd status of the sib-pair at the locus being tested is a significant predictor of the co-sib's score, then there is evidence for linkage (Plomin, DeFries, McClearn, & Rutter, 1997). Figure 2 illustrates the predictions for the co-sib's score based on the sib-pair's ibd status, assuming there is evidence for linkage. In the figure, P stands for proband and C stands for co-sib.

Figure 2. An illustration of the DeFries-Fulker linkage method.



The DF basic equation is given below:

$$C = B_1P + B_2\hat{\pi} + K$$

C represents the co-sib's phenotypic score, P represents the proband's phenotypic score, and $\hat{\pi}$ is the estimated ibd of the sib-pair. This equation was used to establish evidence for linkage before proceeding with tests of G x E interaction. Evidence of linkage is given by the significance of the B_2 term. A one-tailed test of B_2 is customarily used to test for linkage because the direction of regression to the mean is nearly certain (e.g., DeFries et al., 1987).

The extended form of the DF equation (Fulker et al., 1991) can incorporate a G x E interaction term:

$$C = B_1P + B_2\hat{\pi} + B_3e + B_4Pe + B_5\hat{\pi}\,e + K$$

As before, C represents the co-sib's phenotypic score, P represents the proband's phenotypic score, and $\hat{\pi}$ is the estimated ibd of the sib-pair. The new term, "e" represents a pair-specific or person-specific environmental variable. The beta weight of interest in

this equation is B_5, which gives an estimate of the significance of the G x E interaction. A two-tailed test of B_5 was employed because the direction of the interaction could theoretically be in either direction. Before running the DF extended model, the variables were centered and interaction terms were computed from these centered variables.

All DF regression models were performed with SPSS 16.0. A selection criteria of 1.5 SD below the comparison sample mean was used to select probands for all analyses. Because the sample was truncate selected, sib-pairs in which both members met the extremity selection criteria (1.5 SD below the comparison mean) were double-entered (DeFries & Gillis, 1991). The standard errors of the regression coefficients were corrected for the number of double-entered pairs using the procedures described by Stevenson et al. (1993), which is a conservative correction (Kohler & Rodgers, 2001; Rodgers & Kohler, 2005).

HE methods use the general approach of regressing Y, which is a function of the traits y_1 and y_2 of the members of the sib-pair, on $\hat{\pi}$, the estimated ibd status of the sib-pair:

$$Y = B_1\hat{\pi} + K$$

If $\hat{\pi}$ significantly predicts the concordance of the phenotype between the sib-pairs (e.g., $\hat{\pi} = 0$ is associated with discordant phenotypes and $\hat{\pi} = 1$ is associated with concordant phenotypes), then there is evidence for linkage. The particular function Y that maximizes the power of the method has been the subject of many recent reports (e.g., Drigalenko, 1998; Elston et al., 2000; Forrest, 2001; Sham & Purcell, 2001; Visscher & Hopper, 2001; Wright, 1997; Xu et al., 2000). Although each approach has strengths and

limitations, the mean–corrected cross-product of the sib-pair's phenotypes was used in this study because it is robust to distributional assumptions, which is an important consideration in this selected sample (Elston et al., 2000). All HE analyses for this study were conducted using the S.A.G.E software package.

This study used the following specific equation for the HE linkage analysis:

$$C \cdot P = B_1 \hat{\pi} + K$$

$C \cdot P$ is the mean-corrected cross product of the proband's and co-sib's phenotypic score and $\hat{\pi}$ is the estimated ibd of the sib-pair. It is important to note that the distinction between co-sib and proband is arbitrary in the HE analysis because there is no selection criteria for HE. Instead, all possible sibling pairs are entered into the analysis. In families with multiple sibships, simulations have shown that each sib-pair can be treated independently as long as there is an adequate number of sibships in the whole sample (Blackwelder & Elston, 1985). In HE analysis, evidence of linkage is given by a two-tailed significance test of the B_1 term.

The HE equation has been adapted to test for G x E interactions:

$$C \cdot P = B_1 \hat{\pi} + B_2 e + B_3 \hat{\pi} e + K$$

As before, $C \cdot P$ is the mean-corrected cross product of the proband's and co-sib's phenotypic score (but note that these designations are arbitrary in HE analysis) and $\hat{\pi}$ is the estimated ibd of the sib-pair. The new term, "e" is a pair-specific environmental measure. Person-specific environmental variables cannot be tested in this model. The

beta weight of interest in this equation is B_3, which gives an estimate of the significance of the G x E interaction term (Gauderman et al., 2001; Schaid et al., 2003). A two-tailed test of B_3 was employed because the direction of the interaction could theoretically be in either direction.

Chapter 3

Results

The results will focus on four primary issues (1) identification of significant linkage peaks in four replicated linkage regions of RD on chromosomes 1, 3, 6, and 15, (2) examination of main effects of home environmental and bioenvironmental variables, (3) investigation of G-E correlations for those environmental variables demonstrating main effects, and (4) analysis of G x E interactions at the identified linkage peaks with environmental variables showing main effects.

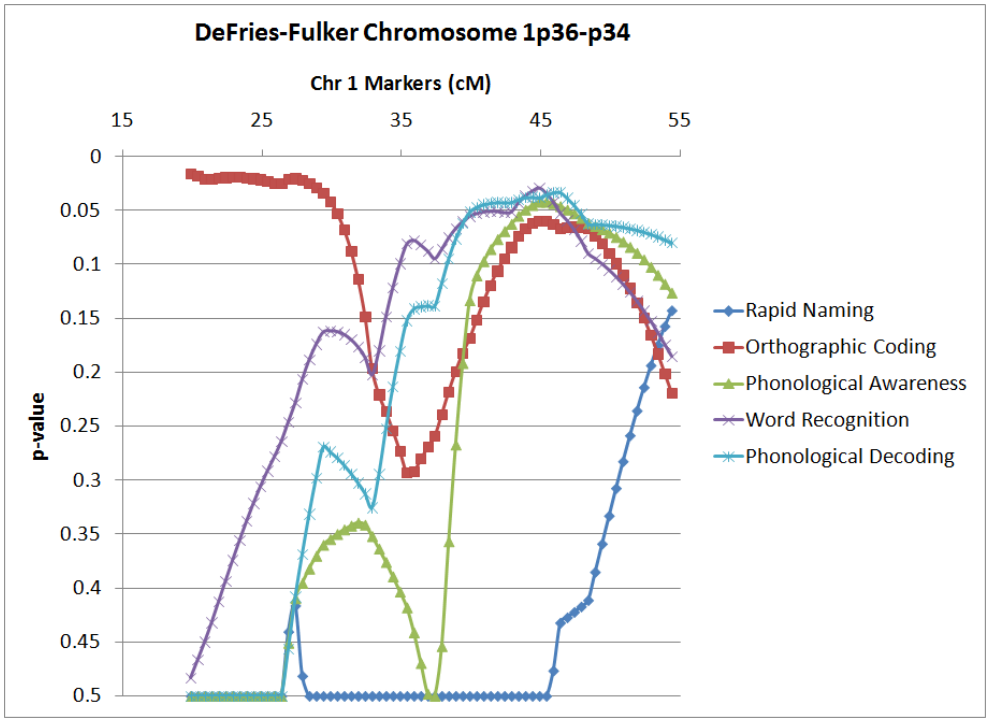
Linkage Analyses.

As discussed in the analysis section of the Methods, two regression-based linkage approaches were employed, DeFries-Fulker (DF) and Haseman-Elston (HE) methods. The DF method was considered to be the primary analysis because it has been shown in simulations to be more powerful in samples selected for extremity (Lessem et al., 2001). However, HE methods are widely used and well-characterized, and so it was important to conduct secondary analyses to assess convergence with DF methods (Feingold, 2001). There is much discussion in the literature regarding appropriate corrections for multiple testing in linkage analyses (e.g., Chen & Storey, 2006; Lander & Kruglyak, 1995).

Standard corrections, such as the Bonferroni correction, are too conservative as the statistical tests are not independent because the phenotypes are highly correlated and the markers are tightly linked. Although correction methods have been developed for genome-wide linkage analyses (e.g., Chen & Storey, 2006), this study was targeting previously identified RD linkage peaks. As a result, the alpha level for a significant linkage signal was adjusted to $p<.01$ based on recommendations by Lander and Krugylak (1995) for replicating a linkage result. In addition, I noted trends with significance values of $p<.05$ because the DF correction for lack of independence of double-entered sib pairs is overly conservative (Kohler & Rodgers, 2001; Rodgers & Kohler, 2005).

Results for the multipoint DF and HE linkage analyses are depicted in Figures 3, 4, 5, and 6 below. Both graphs show the p-value associated with the linkage result for each multipoint interval. On chromosomes in which candidate genes have been identified, the location of the genes is indicated for comparison with the obtained linkage peaks.

Figure 3. Linkage of chromosome 1 markers and literacy phenotypes using DeFries-Fulker and Haseman-Elston linkage methods. The significance of linkage (*p* value) is graphed against the chromosomal position of the markers on 1p36-p34.



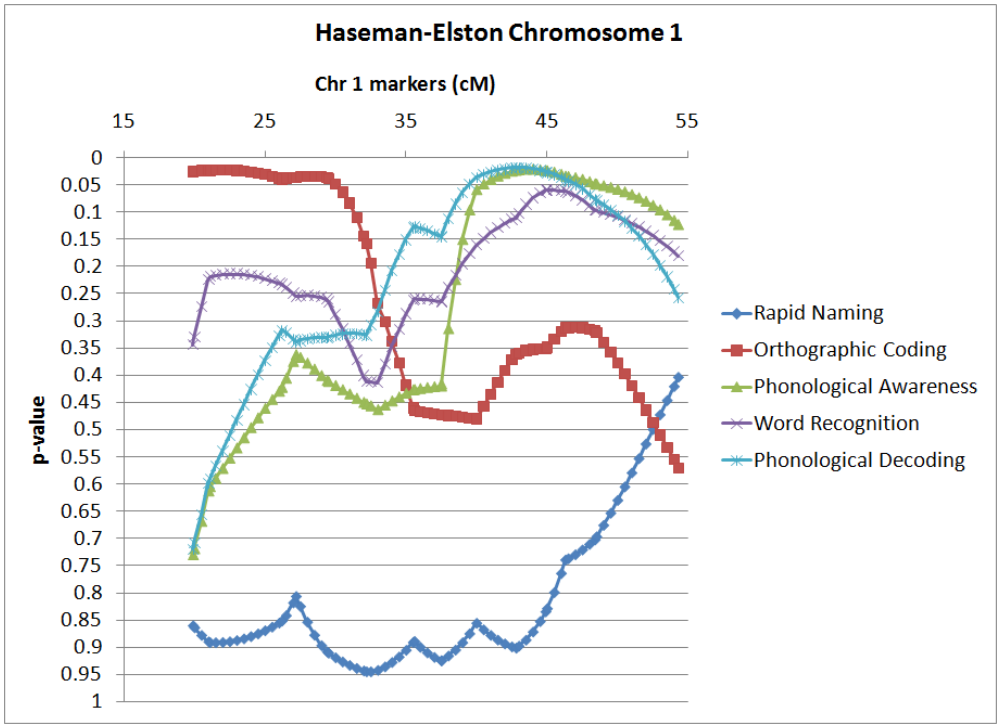
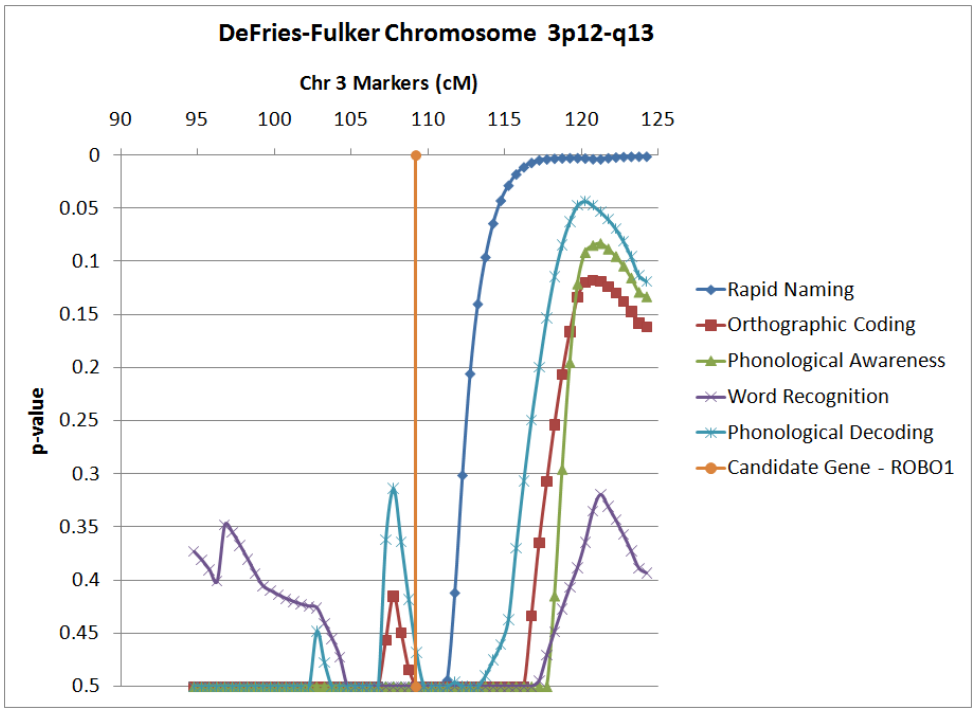


Figure 4. Linkage of chromosome 3 markers and literacy phenotypes using DeFries-Fulker and Haseman-Elston linkage methods. The significance of linkage (*p* value) is graphed against the chromosomal position of the markers on 3p12-q13. The location of the candidate gene ROBO1 is also depicted on the graph.



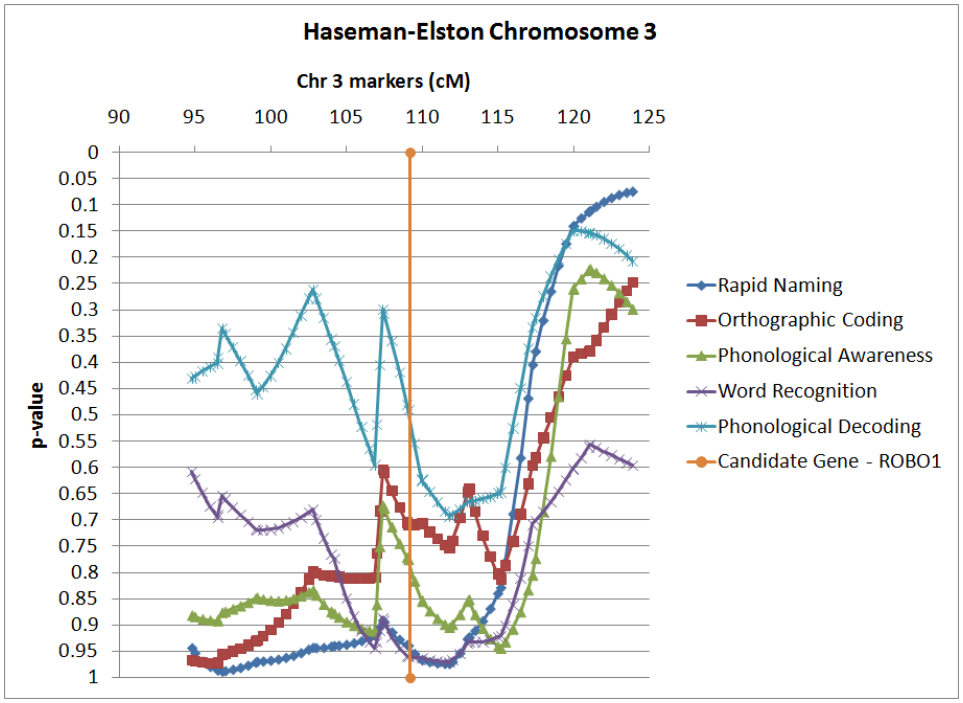


Figure 5. Linkage of chromosome 6 markers and literacy phenotypes using DeFries-Fulker and Haseman-Elston linkage methods. The significance of linkage (*p* value) is graphed against the chromosomal position of the markers on 6p22.2. The location of the candidate genes DCDC2 and KIAA0319 are also depicted on the graph.

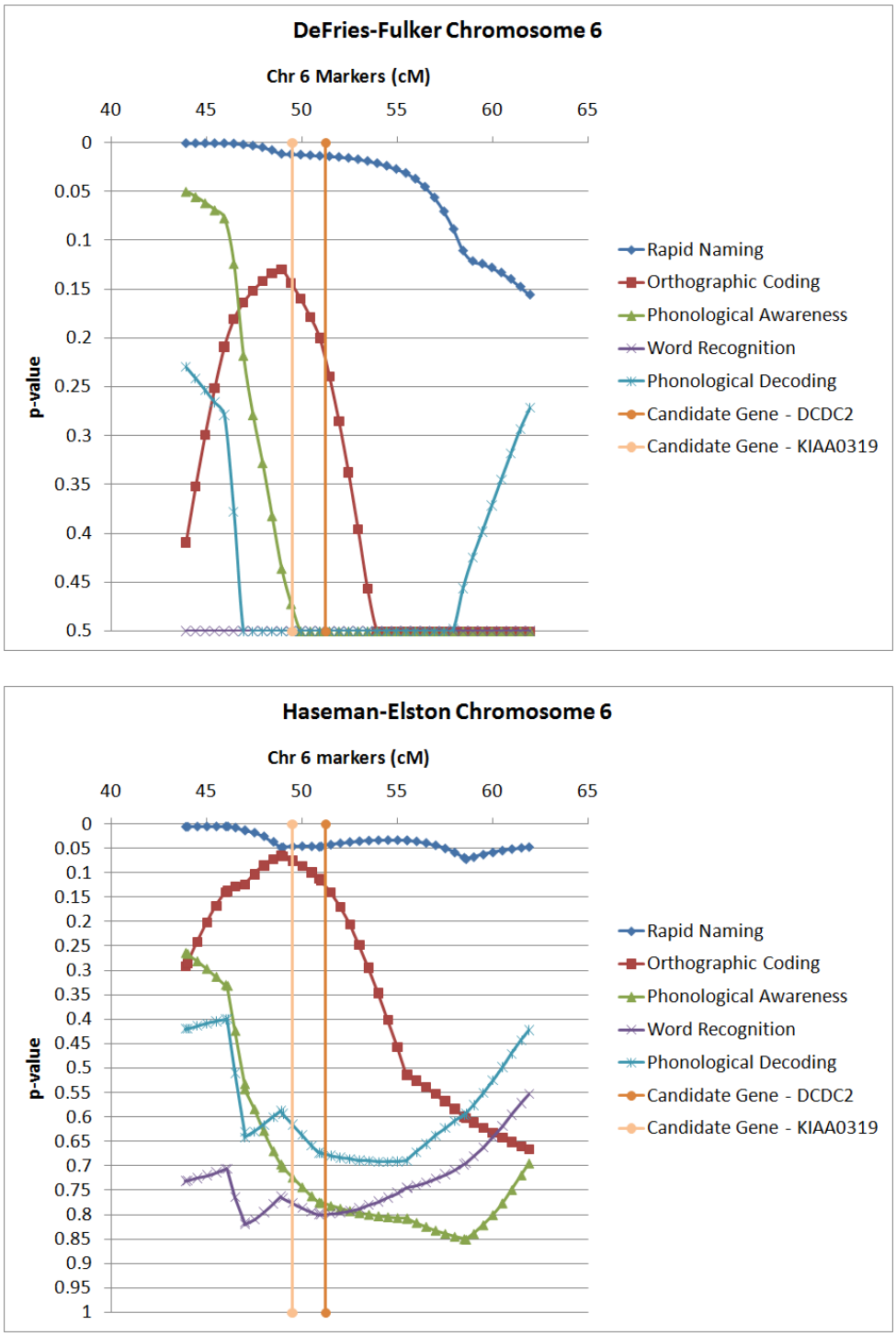
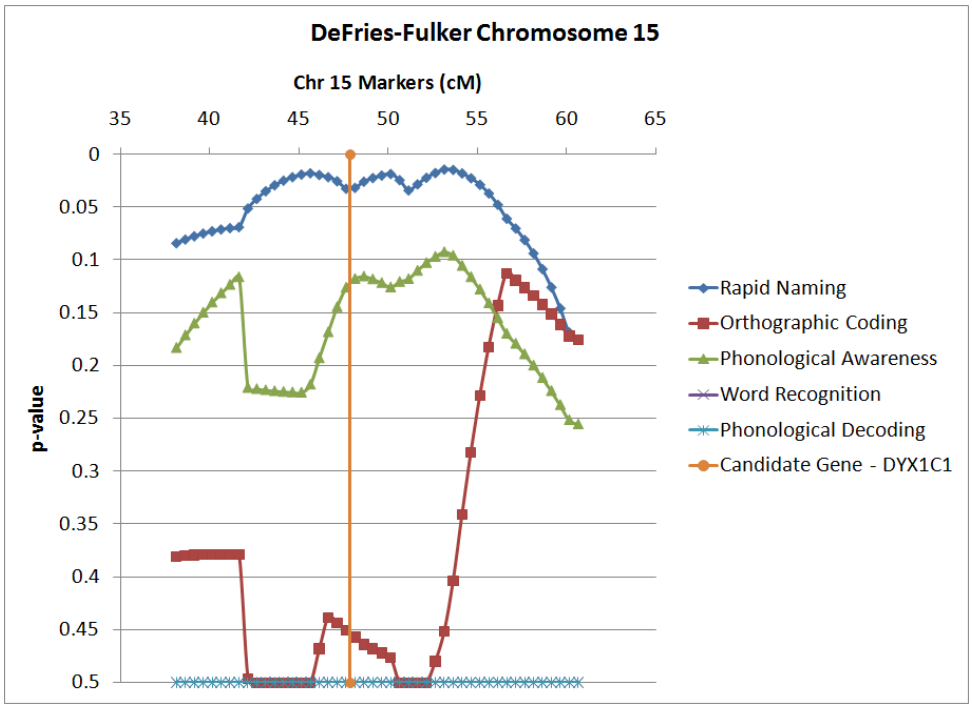
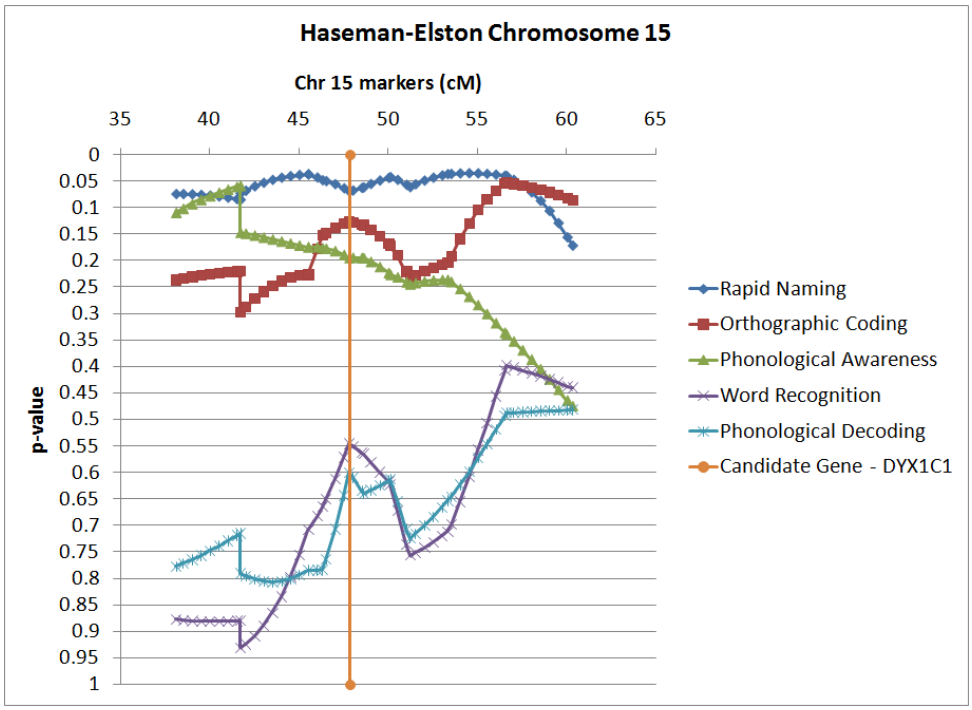


Figure 6. Linkage of chromosome 15 markers and literacy phenotypes using DeFries-Fulker and Haseman-Elston linkage methods. The significance of linkage (*p* value) is graphed against the chromosomal position of the markers on 15q21. The location of the candidate gene DYX1C1 is also depicted on the graph.





The goal of the linkage analyses was to identify linkage peaks that showed convergence across methods and phenotypes so that they could be included in further models incorporating tests of G x E interaction. Trend-level linkage peaks were noted because G x E interactions can suppress the overall linkage signal when the linkage is moderated by the environmental context.

Overall, the two linkage methods showed good correspondence in terms of overall morphology of the graphs and specific phenotypes reaching significance and showing trends. The linkage peaks also showed good correspondence with the locations of the proposed candidate genes, with minor displacements likely due to variations in the informativeness of the markers. Regarding specific phenotypes, only RN reached the significance cut-off of $p < .01$ in the DF and HE analyses. In the DF analysis, RN showed a significant linkage peak on chromosome 3 at 120 cM and on chromosome 6 at 44 cM. In the HE analysis, there was converging evidence for a significant linkage peak for RN on chromosome 6. At the chromosome 3 location, the RN phenotype did not reach trend-level significance ($p = .074$), but the morphology of the phenotype was similar to the DF analysis.

There were also additional trends with each of the other phenotypes. On chromosome 1, DF analysis showed trend-level peaks for PA, Wrec, and PD at 44 cM. There was correspondence for each of these trend peaks from the HE analysis, except for the Wrec peak which was just above trend level cut-offs, $p = .059$. In both analyses, there was a separate linkage peak at 20 cM for OC. Because this peak did not show convergence from other phenotypes and was distal from the linkage peak at 44 cM, it was

not included in further analyses. On chromosome 3, the DF analysis showed a trend-level linkage peak for PD at 120 cM. The same morphology of the phenotype was evident in the HE analysis, although the PD phenotype did not reach trend-level significance (p =.15). On chromosome 6, the DF analysis showed a trend-level linkage peak for PA at 44 cM that did not receive convergence from the HE analysis (p =.26). On chromosome 15, there was convergence from both the DF and HE analysis showing a trend for the RN phenotype at 53 cM.

Overall, there was evidence for significant linkage of the RN phenotype at chromosome 3 and chromosome 6 markers, and there were trend-level linkage peaks for the other literacy phenotypes at each of the four chromosome locations. These results are consistent with previous linkage analyses in RD. There was also good correspondence between the two analytic approaches. Consistent with simulations suggesting higher power for the DF analysis in selected samples, there were no significant linkage peaks in the HE analysis that were not identified by the DF analysis. Instead, patterns across the two analytic approaches suggested that the DF analysis was more sensitive, but there was generally corroboration from the HE analysis, albeit at a weaker level of significance. Linkage peaks within each analytic strategy that showed correspondence across phenotypes were selected for further analysis in G x E interaction models. These phenotypes are listed in Table 10 below. The $B_2\hat{\pi}$ term is given for the DF analysis because this term represents the heritability (h^2_g) of the QTL as a result of linear transformations of the data that were performed prior to the analysis (DeFries & Fulker, 1988).

Table 10. Correspondence of linkage results for DF and HE analytic approaches. Phenotypes listed were selected for further analysis in G x E interaction models. Shaded rows indicate that the phenotype did not reach trend level significance in the analysis.

DeFries-Fulker					Haseman-Elston		
Chromosomal location	Phenotype	$B_2\hat{\pi}$	(SE)[1]	p-value	Chromosomal location	Phenotype	p-value
chr 3 – 120.26 cM	RN	.347	0.124	p<.01			
chr 6 – 44.43 cM	RN	.418	0.129	p<.01	chr 6 – 45.5 cM	RN	p<.01
chr 1 – 44.38 cM	PA	.253	0.149	p<.05	chr 1 – 44.0 cM	PA	p<.05
chr 1 – 44.38 cM	Wrec	.203	0.109	p<.05			
chr 1 – 44.38 cM	PD	.192	0.107	p<.05	chr 1 – 44.0 cM	PD	p<.05
chr 3 – 120.26 cM	PD	.193	0.112	p<.05			
chr 6 – 43.93 cM	PA	.269	0.163	p<.05			
chr 15 – 53.12 cM	RN	.275	0.124	p<.05	chr 15 – 54.5 cM	RN	p<.05

[1] Standard errors are corrected for double-entry.

Environmental Main Effects

A conservative approach to the G x E interaction analysis was taken by limiting the analyses to environmental variables that showed main effects on the child's single word recognition skills (Wrec). Since this study was considering a wide array of home and bioenvironmental variables, I chose to focus on variables showing main effects in order to reduce the potential for Type I error. Each environmental variable was tested for its relationship with Wrec. This phenotype was chosen because it is the definitional core of RD. Results of the tests for main effects of the environmental variables are reported in Table 11.

Table 11. Main effects of environmental variables. When group comparisons are presented, the dependent variable is expressed as a standardized score relative to the comparison sample mean and SD. For all analyses, one child from each family was randomly selected except in the case of child-specific variables that differed between twins (e.g., print exposure, birth weight). Highlighted rows indicate significant results.

Potential Moderators	Less Optimal Environment M (SD)	Enriched Environment M (SD)	Results
General Home Environment			
Parent Education			$r(1793) = .359$, $p<.001$
Family Size			$r(1819) = -.028$, *ns*
Birth Order			$r(1819) = -.043$, *ns*
HLQ Family's Estimated Annual Income			$r(103) = .299$, $p<.01$
HLQ Attended educationally-focused preschool (twins only)	-1.27 (1.24)	-1.01 (1.49)	$t(82) <1$, *ns*
Home Literacy Environment			
			$r(108) = .203$, $p<.05$
Mother Home Literacy Activities			$r(1374) = .098$, $p<.001$
Father Home Literacy Activities			$r(1261) = .132$, $p<.001$
Books in Home			$r(1391) = .226$, $p<.001$
Child Print Exposure			$r(3114) = .533$, $p<.001$
Preschool Shared Reading Practices			
HLQ – How often did you read out loud to your twins?			$r(85) = .064$, *ns*
HLQ – How many picture books did you have in your home?			$r(85) = .079$, *ns*
Family Educational Values			
	-1.24 (1.60)	-.65 (1.32)	$t(94) = 1.927$, $p=.057$
HLQ Reading Activity	-.61 (1.42)	-.83 (1.47)	$t(82) <1$, *ns*
HLQ Child responsibility			$r(107) = .015$, *ns*
HLQ Parent responsibility			$r(107) = .101$, *ns*
HLQ Teacher responsibility			$r(107) = -.079$, *ns*
Pre- and Perinatal complications & Biological Risk Factors			
Mother's age at birth of twins			$r(1795) = .234$, $p<.001$
partial mother's education			$r(1779) = .125$, $p<.001$
Birthweight (twins only)			$r(3522) = .095$, $p<.001$
DZ twins only			$r(2038) = .092$, $p<.001$
MZ twins only			$r(1484) = .066$, $p<.05$
Prematurity - weeks premature (twins only)			$r(1654) = -.047$, $p=.054$
Prematurity- categorical (twins only)	-1.15 (1.44)	-.91 (1.49)	$t(1652) = 1.871$, $p=.062$
Prenatal exposure to smoking	-.97 (1.40)	-.48 (1.48)	$t(547) = 2.434$, $p<.05$
Alcohol during pregnancy	-.48 (1.24)	-.54 (1.54)	$t(543) <1$, *ns*
Drugs during pregnancy	-1.12 (1.57)[a]	-.53 (1.48)	$t(539) =1.353$, $p=.177$
Obstetric Complications			$r(474) = .042$, *ns*
Season of birth			$F(3, 1925) = 1.038$, *ns*

[a] sample size of mothers endorsing drug use was small, $n=12$.

The first thing to note about the table is the wide range of sample sizes in each analysis. As discussed in the methods section, some of these environmental variables were collected in the full sample whereas others were collected in only a subsample via a supplementary mailing. Additionally, some of the analyses were only conducted with the twin-pairs only, not with additional sibling pairs. In the case of the variable indexing attendance at educationally-focused preschools, this analysis was conducted in twins only because the data was only collected for twin pairs in the supplementary mailing. However, in the case of the birth weight and prematurity variables, the analyses were conducted within the twin sample only because including the singleton siblings would result in spurious main effects. This is the case because twins are more likely to be premature and have low birth weights compared to singletons. Additionally, because the sample was recruited for affected twins, the siblings of these twins are less likely to have RD based on regression to the mean. Thus, including singletons in the birth weight and prematurity analyses would have resulted in spurious main effects.

The large sample sizes for some of the analyses indicated that it was important to pay attention to the magnitude of the effect, not just the significance value. For example, Mother's and Father's Home Literacy Activities showed a significant relationship with Wrec in the child, but the magnitude of the effect ($r \sim .1$) was much weaker than the other variables indexing the home literacy environment. As a result, these variables were dropped from further analysis in favor of the other more strongly related variables. Similarly, mother's age at birth of twins was dropped from further analyses because the directionality was opposite from predictions. It was hypothesized that older maternal age

at birth would be associated with poorer outcomes based on results reported in other developmental disorders like ADHD and SLI. In this sample, older maternal age was associated with better outcomes. Older maternal age was also associated with higher years of education. When mother's education was partialed from the correlation between maternal age and Wrec, the relationship was no longer as strong, indicating that education partly accounted for the positive relationship between maternal age and reading outcome. Because parent education was already selected as an environmental variable for further study, maternal age was dropped from further analyses because it was not strongly related to Wrec after accounting for education.

Because the list of home environmental variables that showed significant main effects was still quite large, I further reduced the list of variables to test in G x E interaction by examining the strength of the correlation with single word reading skills when parent education or parent reading history was controlled. The purpose of these analyses was to ensure that parent education was not capturing all of the variance in the literacy environment. If so, then one could reasonably test for G x E interaction with parent education only. It was also important to assess whether these environments were being driven primarily by the parents' own reading history, which was indexed by their score on the past items of the RHQ. The parent's score on the past items of the RHQ was used as a proxy for the parent's genetic risk for RD. If the relationship between the home environmental variable and the child's Wrec score was entirely accounted for by the parent's own reading history, then the relationship could be explained by shared genetic risk factors between the parent and the child rather than environmental effects. Such

passive G-E correlations might result in spurious G x E interaction results, a topic that is discussed further below (Purcell, 2002). Thus, home environmental variables that were correlated with the child's Wrec score and were robust to partialing of parental education and parental reading history were selected for further analysis in G x E interaction models. Table 12 reports the results of these partial correlations.

Table 12. Correlations between home environmental measures and single word recognition skills while partialing parent education and parent reading history. Shaded rows indicate variables that were robust to the partial correlations.

Potential Moderators	Results
General Home Environment	
Parent Education	$r(1793) = .359$, $p<.001$
Partial mother and father past RHQ	$r(482) = .202$, $p<.001$
Family's Estimated Annual Income	$r(103) = .299$, $p<.01$
Partial parent education	$r(100) = .231$, $p<.05$
Partial mother and father past RHQ	$r(58) = .092$, *ns*
Home Literacy Environment	
	$r(108) = .203$, $p<.05$
Partial parent education	$r(105) = .149$, *ns*
Partial mother and father past RHQ	$r(60) = .113$, *ns*
Books in Home	$r(1391) = .226$, $p<.001$
Partial parent education	$r(1388) = .077$, $p<.01$
Partial mother and father past RHQ	$r(482) = .166$, $p<.001$
Child Print Exposure	$r(3114) = .533$, $p<.001$
Partial parent education	$r(3071) = .498$, $p<.001$
Partial mother and father past RHQ	$r(1072) = .409$, $p<.001$

Table 13 below reports the correlations between the selected home environmental variables and parent's reading history variables.

Table 13. Correlations between home environmental variables and parent reading history variables.

	Parental education	Mother past RHQ	Father past RHQ	Books in Home	Child Print Exposure
Parental education	1				
Mother past RHQ	.259**	1			
Father past RHQ	.240**	.137**	1		
Books in Home	.450**	.182**	.188**	1	
Child Print Exposure	.259**	.187**	.128**	.194**	1

*p<.05, **p<.01

The previous discussion focused on home environmental variables, but it is also important to consider the inter-relations of the pre- and perinatal risk factors that showed associations with Wrec, birth weight and prenatal exposure to cigarette smoke. Not surprisingly, smoking during pregnancy showed a significant association with birth weight, such that mothers who smoked had infants that weighed less (M=79.74oz, SD=19.70) than mother who did not smoke (M=88.23oz, SD=20.26), $t(469) = 2.808$, p<.01. The dependency between these variables will be considered if the variables show significant G x E interactions.

Overall, the goal of the environmental main effects analyses was to identify home and bioenvironmental risk factors that impacted Wrec. Once these environmental variables were identified, further analyses were conducted to determine the independence of the effects on Wrec. In the case of the home environmental variables, three variables, parent education, books in the home, and child print exposure, showed evidence of statistically independent effects on Wrec when parental reading history or parent education (in the case of books in the home and child print exposure) were taken into

account. In the case of the bioenvironmental risk factors, prenatal exposure to smoking and birth weight both showed significant associations with Wrec. These five home and bioenvironmental variables were included in G x E interaction models at the previously identified linkage peaks. I turn now to testing for G-E correlations in preparation for conducting the G x E interaction analyses.

Gene-Environment Correlations

The analysis of G-E correlations was restricted to the home environmental variables because the genetic risk factors for RD which are the focus of this investigation can be more directly linked to the home environmental variables than the bioenvironmental variables. Passive G-E correlations are an important consideration in genetic designs that use parental environmental variables to predict child outcomes. Evocative and active forms of G-E correlations can also impact the home environment, such that a child's genetic risk factors may impact the ways that parents interact with a child and the kinds of experiences that a child seeks out with a parent.

Testing for the different forms of G-E correlations in the context of a linkage study is difficult because the specific risk alleles are unknown. Thus, I used the measures available to create proxies for the child's and parent's genetic risk factors and then correlated these proxies with the selected environmental variables. To assess passive G-E correlations, the parent's report of their reading history on the RHQ was used as a proxy for the parent's RD genetic background. The child's IQ was also used as a proxy for the parent's IQ to index broader genetic cognitive risks that may impact home

environmental measures. To assess passive, active, and evocative G-E correlations, the child's Wrec score was taken as a proxy for their own RD genetic risk factors. Of course, these methods are imprecise and using the measures as genetic proxies is an over-simplification, especially given our interest in the multifactorial nature of RD phenotypes. However, this approach represents an approximation towards considering G-E correlations in the context of G x E interactions. The correlations between these genetic proxies and the selected environmental measures are presented in Table 14 below. Many of these correlations have been previously presented (e.g., the environmental variables were selected for their correlation with Wrec), but they are presented in this form below to illustrate the potential genetic relationships between these variables whereas previous presentations have emphasized potential environmental relationships.

Table 14. Correlations between genetic proxies and selected environmental measures.

	Mother past RHQ	Father past RHQ	Child IQ	Child Single Word Recognition (Wrec)
Parental Education	.259**	.240**	.383**	.338**
Books in Home	.182**	.188**	.280**	.206**
Child Print Exposure	.187**	.128**	.419**	.533**

*p<.05, **p<.01

These patterns of correlations indicated that there are potential G-E correlations that must be considered when testing for G x E interactions with these environmental variables. Additionally, because child print exposure is a child-specific variable, the twin design of this study allowed a direct estimate of the heritability of this measure to be

calculated in this sample. The heritability estimate was modest but approaching significance, $h^2_g = .14$, SE = .09, $t(427) = 1.554$, $p = .06$, consistent with previous reports in other large twin samples (Harlaar et al., 2007). Overall, there is evidence that the "environmental" variables are partially determined by genetic risk factors that can be passed on to children (passive G-E correlations) who subsequently create and receive their own environments based partially on their genetic endowment (active and evocative G-E correlations). These G-E correlations will be controlled for if significant G x E interactions are detected in order to minimize the risk of spurious G x E interaction results.

Gene x Environment Interactions

The DF extended model was the primary analysis used to test for G x E interactions because it is able to model both pair-specific and child-specific environmental variables (Fulker et al., 1991), whereas HE models can only model pair-specific variables (Schaid et al., 2003). The HE models were used to assess convergence with the DF G x E analysis for pair-specific variables. The results of the DF analyses will be discussed first followed by the HE analyses.

The DF extended model was run at the eight significant and trend-level linkage peaks on chromosomes 1, 3, 6, and 15 (see Table 10) with the five significant home and bioenvironmental variables. A Bonferroni correction for multiple testing is too conservative in this case because the genetic markers within a linkage peak are tightly linked and the phenotypes and environmental variables are correlated. To control for

Type I error, I set the alpha value to $p < .01$ and noted non-significant trends of $p < .05$ because considerable power is necessary to detect interactions and because the double-entry correction is known to be conservative (Kohler & Rodgers, 2001; Rodgers & Kohler, 2005).

Of the 5 home and bioenvironmental variables tests, only parent education showed significant G x E interactions. None of the other environmental variables showed any evidence of G x E interactions, all *p's* >.1. Sample sizes for the home environmental variables, books in the home and child print exposure (N = 146-266), were generally commensurate with the parent education analysis. Sample sizes for the bioenvironmental variables, smoking during pregnancy and birth weight were reduced. For smoking, the sample size was smaller because this question was added more recently to the parent questionnaire battery (N = 88-192). For birth weight, the sample size was reduced (N = 64-147) because the analysis was limited to twin pairs.

Table 15 below presents the results of the DF G x E interaction analyses with parent education. In this table, the sign of the B_5 term indicates the direction of the interaction. Positive terms indicate diathesis-stress interactions whereas negative terms indicate bioecological interactions.

Table 15. DF G x E interaction analyses with parent education.

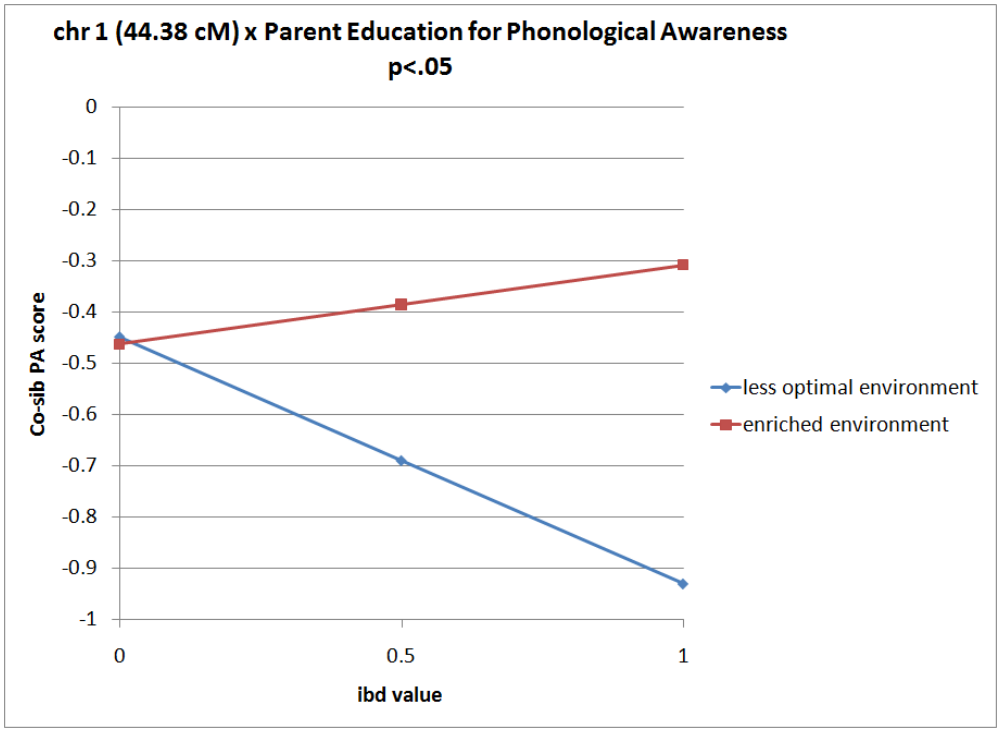
	Phenotype	*N*	$B_5\hat{\pi}$ e	(SE)[1]	Standardized β	*p*-value
p<.01 linkage peaks						
chr3 - 120.26 cM	RN	161	0.076	0.061	0.097	0.2593
chr 6 - 44.43 cM	RN	161	0.025	0.057	0.034	0.6908
p<.05 linkage peaks						
chr 1 - 44.38 cM	PA	186	0.159	0.063	0.179	0.0307
chr 1 - 44.38 cM	Wrec	311	-0.018	0.042	-0.023	0.7167
chr1 - 44.38 cM	PD	304	-0.029	0.042	-0.039	0.5562
chr3 - 120.26 cM	PD	304	0.131	0.043	0.167	0.0098
chr6 - 43.93 cM	PA	186	-0.029	0.066	-0.031	0.7050
chr 15 - 53.12 cM	RN	161	0.119	0.055	0.166	0.0511

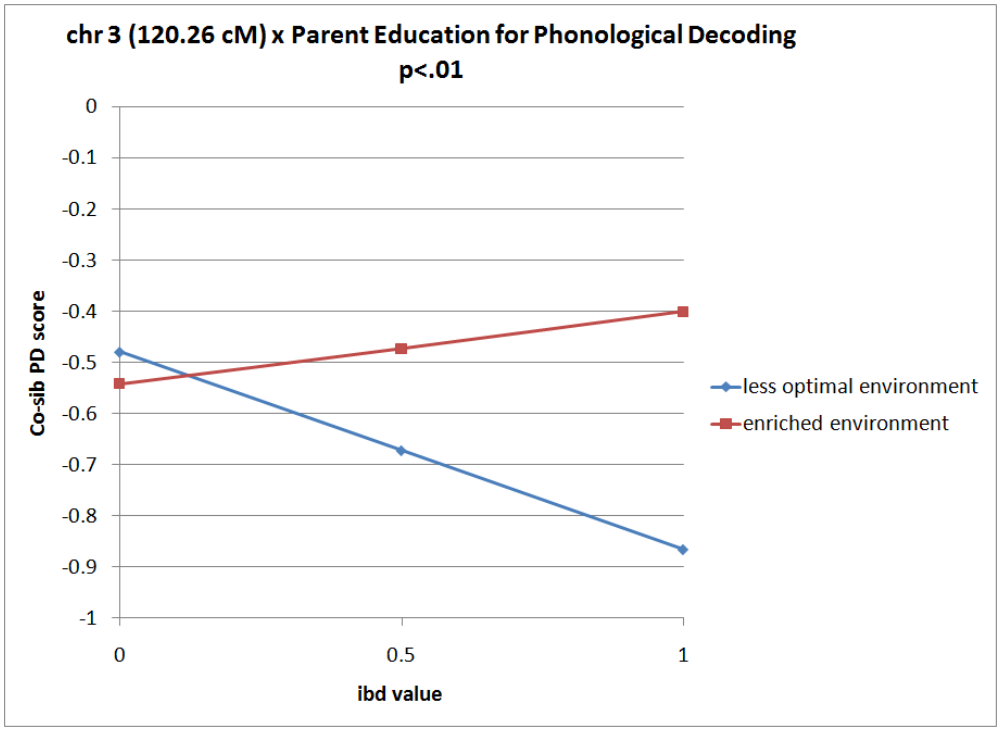
[1] All standard errors are corrected for double-entry.

From this table, it is evident that significant and trend G x E interactions only occurred at the trend-level linkage peaks. This result is consistent with the fact that G x E interactions, if present, can obscure the overall linkage signal. Secondly, contrary to prediction, the G x E interactions that reached significance or trend-level significance were in the diathesis-stress direction on chromosome 1 and chromosome 3. There was also an interaction on chromosome 15 that was approaching a trend. The graphs below plot the significant and trend-level G x E interactions according to recommendations by Aiken and West (1991). Although the interactions were with continuous measures of parental education, Figure 7 dichotomizes the environment for ease of interpretation (less optimal environment is 1 SD below the mean, enriched environment is 1 SD above the mean). In these plots, the co-sib's score (y-axis) is plotted as a function of his/her genetic relationship with the proband at a specific locus (ibd, x-axis). The y-axis is scaled in terms of SD units below the comparison sample mean of 0 and the proband mean is -1. The slopes of the lines reflect the heritability of the QTL, such that a steeper negative

slope reflects a greater heritability. Thus, the diathesis-stress direction of these interactions means that the heritability of the QTL is higher in poorer environments, so the slope of the line corresponding to the less optimal environment is steeper in the negative direction.

Figure 7. Significant and trend-level G x E interactions with parent education from the DF model.





The HE regression framework was used to assess convergence with the DF G x E interaction results. Because the significant and trend-level G x E interaction results in the DF models were restricted to the parental education variable, the HE G x E interaction analyses were limited to this pair-specific variable in order to focus the follow-up analyses. As with the DF models, the HE G x E analyses were conducted with the four significant and trend-level linkage peaks that were identified in the HE linkage analyses shown in Table 10 above. In addition, the G x E analysis was also run on chromosome 3 with the PD phenotype even though this phenotype did not reach trend level significance in the HE linkage analysis (p =.15). This additional analysis was run in order to assess convergence with the significant G x E result in the DF analysis. Table 16 below presents the results of the HE G x E interaction analyses with parent education. The sign of the B_3 term indicates the direction of the interaction. The directionality of the interactions is opposite of the DF models. In the HE models, negative terms indicate diathesis-stress interactions and positive terms indicate bioecological interactions. The reason for this sign difference is inherent in the models (and illustrated in Figure 7 above and Figure 8 below) because significant linkage in DF models is indicated by negative slopes and significant linkage in HE models is indicated by positive slopes.

Table 16. HE G x E interaction analyses with parent education.

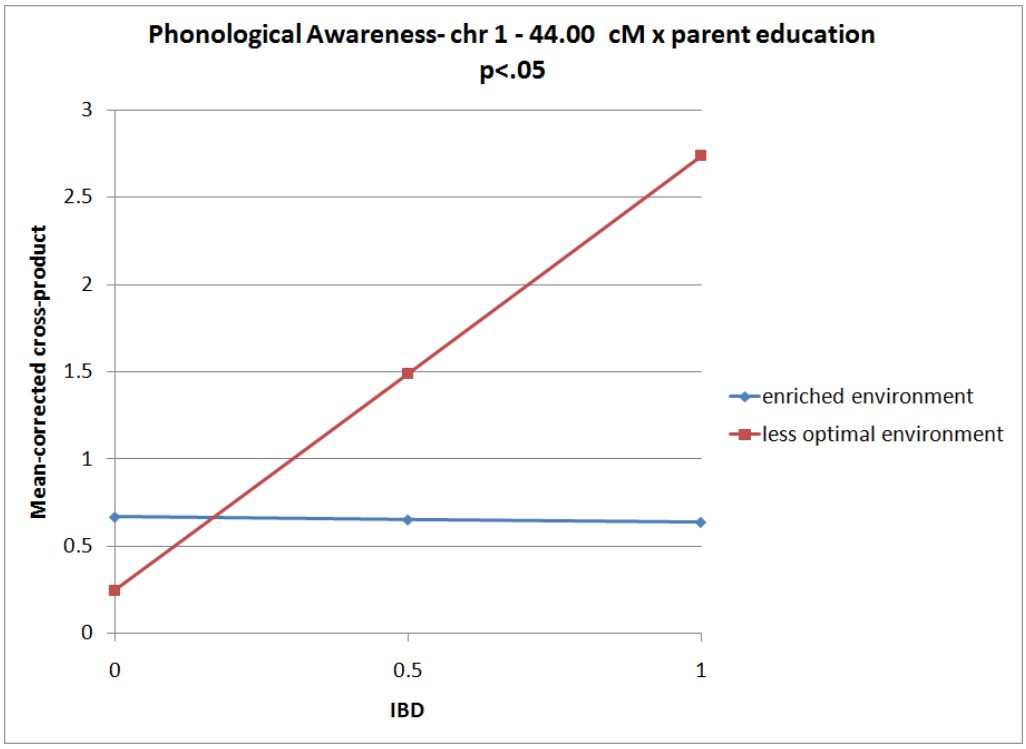
	Phenotype	*N*	$B_3\widehat{\pi}$ e	(SE)	Standardized β	*p*-value
p<.01 linkage peaks						
chr 6 – 45.5 cM	RN	371	-0.066	0.100	-.033	0.517
p<.05 linkage peaks						
chr 1 – 44.0 cM	PA	328	-0.600	0.241	-.135	0.013
chr 1 – 44.0 cM	PD	375	-0.003	0.149	-.001	0.985
chr 3 – 120.0 cM	PD	377	-0.393	0.153	-.133	0.011
chr 15 – 54.5 cM	RN	377	-0.076	0.092	-.043	0.405

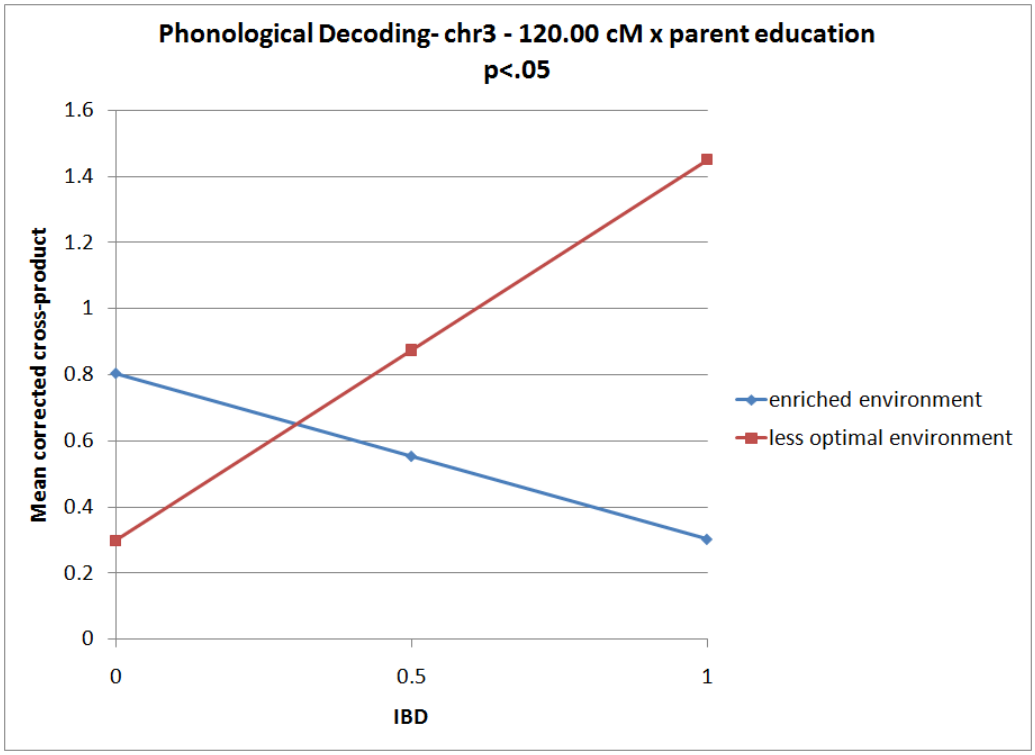
These G x E interactions are convergent with the previous DF G x E results with parent education. As with the DF models, there is evidence for trend-level G x E interactions that are approaching significance on chromosome 1 with PA and chromosome 3 with PD. Both interactions are in the diathesis-stress direction, consistent with the DF results. Because the PD linkage peak on chromosome 3 did not reach significance in the HE linkage analyses (p =.15), it was important to assess the linkage significance of the PD phenotype at chromosome 3 when the G x E interaction was modeled. When the G x E interaction was modeled, the linkage peak at 120 cM increased in significance to p = .004.

The graphs below plot both trend-level G x E interactions according to recommendations by Aiken and West (1991). Although the interactions were with continuous measures of parental education, Figure 8 dichotomizes the environment for ease of interpretation (less optimal environment is 1 SD below the mean, enriched environment is 1 SD above the mean). In these plots, the mean-corrected cross-product of the sibling's phenotypic score (y-axis) is plotted as a function of the siblings' genetic relationship at a specific locus (ibd, x-axis). In these graphs, the slope of the lines

reflects the heritability of the QTL, such that a steeper positive slope reflects a greater heritability. To see why this is the case, consider two sets of siblings that will contribute to a linkage signal, those with convergent phenotypes and ibd 1 or those with divergent phenotypes and ibd 0. For siblings with ibd 1 and convergent phenotypes, suppose that both siblings scored below the mean on the phenotype. In this case, the siblings' mean corrected cross-product will be a large positive number plotted against the ibd value of 1. Compare these two siblings to another set of siblings who are ibd 0 at the locus but have divergent phenotypes such that one sibling is at the mean of 0 and another sibling is below the mean. In this case, the siblings' mean corrected cross-product will be a small number plotted against the ibd value of 0. Thus, the slope of the line will be positive if there is evidence of linkage and flat if there is no evidence for linkage. Figure 8 below plots the relationship between the siblings' mean-corrected cross-product as a function of the ibd of the siblings. Separate lines indicate the moderation effect of the environmental variable, parental education. The diathesis-stress direction of the G x E interactions indicates that the QTL is more heritable in poorer environments, so the slope of the line corresponding to the less optimal environment should be steeper in the positive direction.

Figure 8. Trend-level G x E interactions with parent education from the HE model.





Taken together, the DF and HE G x E analyses showed converging evidence for significant and trend-level diathesis-stress G x E interactions with parent education at chromosome 1 with the PA phenotype and chromosome 3 with the PD phenotype. Follow-up analyses were conducted to examine alternative explanations for these results, including scaling artifacts, G-E correlations, and comorbidities.

Because the interactions were limited to the parental education variable, it was possible that a scaling artifact unique to this variable was responsible for the interactions (Rutter, 1983). The interval scale of the parental education variable is questionable because a one year increase in education could be more meaningful at the lower levels of education than at the higher levels (e.g., 8 versus 9 years of education is a more meaningful increase than 17 versus 18 years of education). As such, the parental education was logarithm transformed to account for this nonlinearity and the G x E interactions were rerun at chromosome 1 with PA and chromosome 3 with PD. Table 17 presents the results of the DF and HE G x E analyses with the log transformed parental education variable as well as the original G x E results with the untransformed variable for comparison.

Table 17. Comparison of G x E results with parent education and logarithm transformed parent education.

	Chr	Location (cM)	Environment	Phenotype	*N*	B $\widehat{\pi}$ e	(SE)	St. β	*p*-value
DF	chr 1	44.38	Parent Ed.	PA	186	0.16	0.06	0.18	0.031
	chr 1	44.38	Log Parent Ed.	PA	186	5.21	2.03	0.18	0.028
HE	chr 1	44.00	Parent Ed.	PA	328	-0.60	0.24	-0.14	0.013
	chr 1	44.00	Log Parent Ed.	PA	328	-22.78	8.19	-0.15	0.006
DF	chr 3	120.26	Parent Ed.	PD	304	0.13	0.04	0.17	0.010
	chr 3	120.26	Log Parent Ed.	PD	304	4.32	1.43	0.17	0.010
HE	chr 3	120.00	Parent Ed.	PD	377	-0.39	0.15	-0.13	0.011
	chr 3	120.00	Log Parent Ed.	PD	377	-13.55	5.19	-0.14	0.009

The results indicate that a scaling artifact in parental education was not responsible for the G x E interactions. When parental education was log transformed, the results remained at the same level of significance and the standardized β estimates remained stable.

The next set of analyses considered possible confounding effects of G-E correlations. Because the interactions were limited to parent education as the environmental variable, these analyses focused on controlling for passive G-E correlations. Active and evocative forms of G-E correlations would have been additional considerations if the books in the home and child print exposure variables had been significant in the G x E interaction analyses because these variables are subject to genetic influences from both the child and the parent. In the case of parent education, passive G-E correlations are the main consideration because most parents have completed their education by the time they have children and so the child's genetic risk factors cannot impact this home environmental variable. Passive G-E correlations were controlled in several different ways to examine the impact of the correlations on the G x E interactions.

First, the parental education variable was residualized for the child's phenotypic score (PA for chromosome 1 analyses and PD for chromosome 3 analyses). Then, this residualized parental education variable was entered as the environmental variable in the G x E interactions. Residualizing the parent education variable controls for the variance that is shared between the parents' education and the child's reading phenotype, which is potentially genetically mediated. The question is whether the parents' education will continue to enter into G x E interactions predicting the child's phenotype after this potential source of genetic variance is controlled. Table 18 below presents the results of the G x E interactions with the residualized parent education variable as well as the original G x E results with the parent education variable for comparison.

A second approach to controlling for passive G-E correlations was to residualize the parent education variable with the parents' reading history as measured by the RHQ. The logic for this analysis was that removing shared variance between the parents' education and reading history would control for the impact of the parents' reading genetic risk on their educational attainment. One complication with this analysis was that only a subsample of the parents received the RHQ and so the sample sizes decreased in these analyses making it difficult to distinguish attenuation of the G x E effects from loss of power due to sample size. Table 18 below presents the G x E analyses with the parent education variable residualized for parents' reading history.

A third approach to controlling for passive G-E correlations was to residualize the parent education variable with the child's IQ. The objective of this analysis was to extend the previous analysis controlling for the child's phenotypic score. This analysis

controls for shared genetic variance related to cognition that may impact both the parent's educational attainment and the child's reading. Table 18 below presents the G x E analyses with the parent education variable residualized for child IQ.

Table 18. Comparison of G x E results with parent education and parent education residualized for child phenotype, parent reading history, or child IQ.

	Chr	Location (cM)	Environment	Phenotype	*N*	B $\widehat{\pi}$ e	(SE)	St. β	*p*-value
DF	chr 1	44.38	Parent Ed.	PA	186	0.16	0.06	0.18	0.031
	chr 1	44.38	Resid. Child phenotype	PA	186	0.32	0.14	0.16	0.052
	chr 1	44.38	Resid. Parent Reading Hx	PA	92	0.21	0.22	0.10	0.414
	chr 1	44.38	Resid. Child IQ	PA	186	0.41	0.15	0.20	0.018
HE	chr 1	44.00	Parent Ed.	PA	328	-0.60	0.24	-0.14	0.013
	chr 1	44.00	Resid. Child phenotype	PA	328	-1.26	0.55	-0.12	0.023
	chr 1	44.00	Resid. Parent Reading Hx	PA	182	-0.43	0.65	-0.05	0.505
	chr 1	44.00	Resid. Child IQ	PA	328	-1.23	0.55	-0.11	0.027
DF	chr 3	120.26	Parent Ed.	PD	304	0.13	0.04	0.17	0.010
	chr 3	120.26	Resid. Child phenotype	PD	304	0.29	0.09	0.17	0.010
	chr 3	120.26	Resid. Parent Reading Hx	PD	127	0.26	0.14	0.15	0.111
	chr 3	120.26	Resid. Child IQ	PD	304	0.29	0.09	0.17	0.008
HE	chr 3	120.00	Parent Ed.	PD	377	-0.39	0.15	-0.13	0.011
	chr 3	120.00	Resid. Child phenotype	PD	377	-0.73	0.34	-0.10	0.033
	chr 3	120.00	Resid. Parent Reading Hx	PD	187	-0.70	0.41	-0.11	0.092
	chr 3	120.00	Resid. Child IQ	PD	377	-0.73	0.34	-0.10	0.032

Overall, the results in Table 18 indicate that the G x E interactions with parent education were robust to controls for passive G-E correlation with a few exceptions. The cases in which the G x E results were not robust were restricted to the analyses with parent education residualized for parent reading history. The sample sizes in these analyses were reduced by about 100-200 sibling pairs, and so a reduction in power may partly explain the lack of robustness. This hypothesis is supported by the standardized β estimates for these analyses, which remained fairly stable in all cases but one (i.e., HE, chr 1, RHQ residualized), despite the drop in the significance of the B term. Besides these exceptions, all of the other approaches to controlling for G-E correlation indicated

that the G x E interactions could not be entirely explained by confounds due to G-E correlation.

The role of comorbid ADHD in this sample was another confound to consider when interpreting the diathesis-stress G x E results. As previously discussed, recent studies have detected diathesis-stress G x E interactions with psychosocial risk factors in ADHD samples (Lasky-Su et al., 2007; Laucht et al., 2007; Retz et al., 2008; Waldman, 2007). Because RD and ADHD commonly co-occur, it is important to examine the potential impact of comorbidity on G x E interactions. To control for the potential confound of ADHD, the child's phenotypic score was residualized for their mean ADHD rating on the 18 symptoms of the DSM-IV ADHD rating scale. This residualized phenotype was then used in the G x E interaction analyses with parent education. The sample sizes for this analysis were reduced because only a subsample of the children were administered the ADHD rating scale. Table 19 below presents the G x E analyses with the residualized phenotypes, as well as the original G x E results with the non-residualized phenotypes for comparison.

Table 19. Comparison of G x E results with the child's phenotype residualized for the child's mean ADHD ratings and the non-residualized phenotype.

	Chr	Location (cM)	Environment	Phenotype	*N*	B$\widehat{\pi}$ e	(SE)	St. β	*p*-value
DF	chr 1	44.38	Parent Ed.	PA	186	0.16	0.06	0.18	0.031
	chr 1	44.38	Parent Ed.	Resid. PA	105	0.16	0.08	0.19	0.079
HE	chr 1	44.00	Parent Ed.	PA	328	-0.60	0.24	-0.14	0.013
	chr 1	44.00	Parent Ed.	Resid. PA	204	-0.30	0.11	-0.20	0.006
DF	chr 3	120.26	Parent Ed.	PD	304	0.13	0.04	0.17	0.010
	chr 3	120.26	Parent Ed.	Resid. PD	147	0.12	0.06	0.16	0.068
HE	chr 3	120.00	Parent Ed.	PD	377	-0.39	0.15	-0.13	0.011
	chr 3	120.00	Parent Ed.	Resid. PD	209	-0.16	0.09	-0.09	0.099

Overall, the results in Table 19 indicate that the DF G x E interactions were generally robust to controls for comorbid ADHD. However, the HE analyses became more unstable when the phenotypes were residualized for ADHD, such that the chromosome 1 G x E interaction result became stronger and the chromosome 3 G x E interaction result became weaker. It is difficult to interpret the significance values of these analyses because of the decrease in power resulting from the reduced sample sizes. Nevertheless, the standardized β estimates were stable in the DF analysis although they were more variable in the HE analysis.

Overall, follow-up analyses of the diathesis-stress G x E analyses indicated that the interactions were generally robust to scaling artifacts as well as controls for G-E correlations and comorbid ADHD. Importantly, there was no evidence for bioecological G x E interactions in any of the primary and follow-up analyses across both the DF and HE methods. Thus, the results were in an unpredicted direction and were divergent from behavioral genetic results that have been obtained in this same sample (Friend et al., 2008). Because only a subsample of the twins in the behavioral genetic analysis had molecular genetic genotypes, I considered the hypothesis that the genotyped subsample of twins may have differed by chance on dimensions that may be important for directionality in G x E interactions. If so, then divergent results could be obtained in the molecular genetic and behavioral genetic analyses. Table 20 presents a comparison of the DZ twins who were genotyped and ungenotyped for a number of descriptors.

Table 20. Comparison of DZ twins with genotypes and without genotypes.

	DZ Genotyped (N = 173 twin pairs)	DZ Ungenotyped (N = 515 twin pairs)	Statistic
Full-Scale IQ	105.08 (11.87)	106.72 (13.23)	$t(2110) = 2.169$, $p<.05$
Age	10.57 (2.30)	11.66 (2.71)	$t(2110) = 7.100$, $p<.001$
Wrec	-1.14 (1.42)	-0.81 (1.45)	$t(2110) = 3.833$, $p<.001$
Parent Education	14.95 (2.18)	14.59 (2.30)	$t(2076) = 2.689$, $p<.01$
ADHD Mean Ratings	0.95 (.68)	0.82 (.66)	$t(1013) = 2.519$, $p<.05$
ADHD Diagnosis	28.2% ADHD	23.4% ADHD	χ^2 (1, N= 1015) = 2.049, p=.172
Gender	53.4% male	50.9% male	χ^2 (1, N= 2112) = .768, *ns*

Although there were several significant results in the comparisons between the DZ genotyped and DZ ungenotyped samples, the magnitude of the differences was quite small. These small differences between the two samples seemed unlikely to result in opposite forms of G x E interactions in the behavioral genetic and molecular genetic analyses.

To further examine the conflicting results between the molecular and behavioral genetic G x E results, a behavioral genetic analysis was conducted comparing the MZ twins (N = 457 twin pairs) to the DZ twins who were genotyped (N = 173 twin pairs) versus those who were not genotyped (N = 515 twin pairs). This analysis explored whether the sample differences reported above, as well as unexamined differences on other dimensions, were accounting for the differing behavioral and molecular genetic G x E results. The DeFries-Fulker behavioral genetic model was used to conduct the analyses (DeFries & Fulker, 1985, 1988). All procedures in the analysis were conducted in accordance with the methods described for the DF linkage models in the current study (e.g., standardization relative to the comparison sample mean and SD, 1.5 SD selection criteria, double-entry correction). The behavioral genetic G x E analysis was conducted with the phenotypes that showed significant or trend-level linkage peaks in the linkage

analysis: RN, PA, Wrec, and PD. As in the molecular genetic G x E analysis, parental education was the environmental factor.

In the analysis with only the genotyped DZ twins, there was a significant G x E interaction term for the PA composite, B= -.129, SE = .065, $t(334) = 1.978$, $p < .05$. There were also nonsignificant trends in the G x E interaction term for the RN, B= -.116, SE = .070, $t(288) = 1.671$, $p < .1$, and Wrec composites, B= -.058, SE = .040, $t(699) = 1.493$, $p = .14$. In the analysis excluding the DZ twins who were genotyped, there was also a nonsignificant trend in the G x E interaction term for the Wrec composite, B= -.049, SE = .029, $t(1042) = 1.679$, $p < .1$. All of these interactions were in the bioecological direction (i.e., negative values), such that the phenotype was more heritable in a more enriched environment, consistent with previous findings in this sample (Friend et al., 2008). For those interactions that did not reach trend or significance levels, there was still a fairly consistent pattern for the terms to be in the bioecological direction (i.e. negative values). Table 21 presents the standardized β estimates for each of the analyses. Overall, the behavioral genetic analyses in both subsamples provided evidence for bioecological G x E interactions, consistent with previous findings in this twin sample (Friend et al., 2008) and others (Kremen et al., 2005).

Table 21. Standardized β estimates for the G x E (parent education) term in the behavioral genetic analysis; (-) interaction terms indicate bioecological interactions, (+) interaction terms indicate diathesis-stress interactions.

	MZ-DZ genotyped	*p*-value	MZ *N* – DZ *N* twin pairs	MZ-DZ ungenotyped	*p*-value	MZ *N* – DZ *N* twin pairs
RN	-.107	*p*<.10	(229-65)	-.010	*ns*	(229-210)
PA	-.117	*p*<.05	(248-92)	-.014	*ns*	(248-243)
Wrec	-.060	*p*=.14	(560-145)	-.055	*p*<.10	(560-488)
PD	.013	*ns*	(471-148)	-.023	*ns*	(471-420)

Summary

In summary, the goal of these analyses was to identify genetic risk factors and environmental risk factors in order to test for possible G x E interactions in RD. A conservative approach to these analyses was taken such that main effects of genes and environments were identified before the interactions were tested. First, sib-pair linkage analyses with both DF and HE models showed evidence of linkage in regions previously associated with RD, 1p36-p34, 3p12-q13, 6p22.2, and 15q21. Secondly, three home environmental variables (parental education, books in the home, and child print exposure) and two bioenvironmental variables (prenatal exposure to smoking and birth weight) showed main effects on child reading. I tested for and carefully considered the possible confounding effects of passive, active, and evocative G-E correlations on the home environmental variables. Initially, DF G x E analyses were conducted with all of the identified genetic and environmental risk factors. From these analyses, only parent education showed significant or trend-level G x E interactions. Follow-up HE analyses showed converging evidence for diathesis-stress G x E interactions with parent education at the chromosome 1 locus with PA and the chromosome 3 locus with PD. Follow-up analyses to control for scaling artifacts, G-E correlations, and ADHD comorbidity revealed that the G x E interactions were generally robust to these confounding factors. Nevertheless, the fact that the interactions were robust and in the diathesis-stress direction created a puzzle since behavioral genetic analyses in this same sample had detected bioecological interactions with parent education. To understand these conflicting results, I conducted behavioral genetic analyses in the subsample of children

with genotypes that were included in the molecular genetic analyses and those that were not included in the molecular genetic analyses. Consistent with previous findings, there was evidence for bioecological G x E interactions in both subsamples despite the reduced sample sizes. Additional exploration of these conflicting behavioral genetic and molecular genetic results will be included in the discussion along with exploration of the possible significance of these diathesis-stress G x E interactions in light of previous molecular genetic research findings.

Chapter 3

Discussion

This study examined G x E interactions in RD using molecular genetic methods and psychosocial and bioenvironmental risk factors. Converging evidence from both DF and HE linkage models indicated significant and trend G x E interactions at the chromosome 1 and 3 loci with parent education as the environmental variable. These interactions were in the diathesis-stress direction, which was contrary to predictions derived from behavioral genetic results in the same sample and molecular genetic results in a related disorder, SSD. The G x E interactions were generally robust to controls for scaling artifacts, G-E correlations, and ADHD comorbidity. In what follows, I will first discuss the linkage and environmental analyses that preceded the G x E interaction analyses, and then discuss the G x E interactions along with explanations for the unpredicted directionality of the interactions.

Linkage Results

The first thing to note about the linkage findings is that it was difficult to detect linkage in this sample. The homogeneity of the phenotypes seemed to be particularly important in the linkage analyses. The initial approach to the creation of phenotypes was to use multivariate composite phenotypes. However, data reduction techniques like EFA

emphasize the shared variance between variables and can result in composites that are theoretically heterogeneous. Although our EFA composites seemed theoretically coherent, these phenotypes did not perform as well in linkage analyses as phenotypes composed of two highly correlated tasks within our specified theoretical domains. Even though the correlations between the EFA composites and the two-variable composites were quite high (*r's* ~ .8 - .9), the linkage results differed for the two sets of phenotypes. This pattern suggests that unshared variance between variables can often be meaningful for genetic analyses. Further evidence for the genetic importance of unshared variance between variables was obtained by Samuelsson et al. (2005) in a behavioral genetic analysis of reading phenotypes. In this study, the authors conducted an exploratory factor analysis of reading phenotypes and formed several composites based on the results of the factor analysis. Several of the factors were further decomposed into subcomponents based on theoretical distinctions within the factors. In some cases, the subcomponents showed statistically significant differences in the magnitude of the genetic and shared environmental influences even though they were derived from the same factor (Samuelsson et al., 2005). These results highlight the importance of using theoretically homogenous phenotypes in genetic analyses.

Multivariate latent traits that model the shared variance and error variance among variables may also help to improve the power of genetic analyses (Marlow et al., 2003; Monaco, 2007). To be maximally informative for genetic studies of developmental disorders, these SEM models will need to be adapted for selected samples where the distributional assumptions are often violated. Such models have been developed for QTL

mapping (Hawke, Stallings, Wadsworth, & DeFries, 2008), but they have not yet been adapted for tests of G x E interaction.

The RN phenotype showed the strongest evidence of linkage in these analyses. Although this phenotype has been included in previous linkage analyses, it is more typical for genetic linkage analyses in RD to emphasize phonological and orthographic phenotypes (Pennington et al., in press). These results suggest that RN phenotypes should continue to be explored in future linkage studies of RD.

One parsimonious explanation for the effectiveness of the RN phenotype in the linkage analyses might be that it was more reliably measured than the other phenotypes. To examine this hypothesis, I computed intraclass correlations in MZ twins as a proxy for reliability. The correlations presented in Table 22 below suggested that all of the phenotypes showed good reliability, but RN did not exceed the others as would be expected if reliability alone could account for the findings.

Table 22. Intraclass correlations of phenotypes within MZ twins as a proxy for reliability.

	RN	PD	Wrec	PA	OC
RN	.621				
PD		.755			
Wrec			.840		
PA				.751	
OC					.668

The RN phenotype is of considerable interest in RD because of recent findings identifying processing speed as a potential shared cognitive deficit between RD and ADHD that may explain the comorbidity between these two disorders (McGrath et al., in

preparation; Shanahan et al., 2006). In these analyses, RN is one of several variables that compose a processing speed factor. Additionally, Cholesky decomposition analyses have shown that processing speed accounts for the genetic relationship between RD and ADHD (Betjemann et al., in preparation). So, processing speed appears to be a key phenotype for understanding the comorbidity between RD and ADHD. Although this project focused on RD, it is important to consider the high rates of comorbidity with ADHD in this sample. Because co-occurring ADHD was not excluded in this sample, it is not surprising that a phenotype which may underlie liability for RD and ADHD would show strong evidence of linkage. The RN phenotype may be a candidate endophenotype in RD and ADHD, which is closer to the mechanisms of gene action and contributes to the behavioral manifestations of both RD and ADHD (Gottesman & Gould, 2003).

Another important point about these linkage analyses is that there was generally impressive correspondence between the DF and HE methods in terms of overall results. Despite this correspondence, it was also evident that the DF method was more powerful for this selected sample, consistent with unpublished simulation results (Lessem et al., 2001). HE regression methods are well-characterized and widely used (Feingold, 2001), but they are not optimal for selected samples. In contrast, DF linkage methods have not been widely disseminated. One contribution of these results is to demonstrate the correspondence between mainstream HE methods and the lesser-known, but more powerful DF methods. The DF linkage model is specifically developed for selected samples and can flexibly incorporate moderators, such as G x E interactions.

Environmental Main Effects

Several of the home environmental variables showed main effects on the child's reading, consistent with the literature on psychosocial influence on reading (Phillips & Lonigan, 2005). Interestingly, the preschool variables indexing shared reading were not significantly related to the child's reading outcome. This finding is difficult to interpret due to the small sample size resulting from the supplementary mailing and the retrospective nature of the parent's report. However, the findings could also be consistent with the small effect sizes reported previously in the literature for shared reading effects on reading outcome (Scarborough & Dobrich, 1994b).

Pre- and perinatal risk factors have been relatively neglected as environmental risk factors in RD, despite active investigation of these factors in comorbid disorders, such as SLI and ADHD. This study detected main effects of birth weight and prenatal smoking exposure on single word recognition skills. Both of these risk factors have been implicated in ADHD and so in follow-up analyses, I covaried the child's mean ADHD score to see if the main effect on reading remained significant. In both cases, the main effect remained significant. These results suggest that further investigation of bioenvironmental risk factors in RD is warranted, especially given that the identified susceptibility genes for RD are implicated in early brain developmental processes.

Gene x Environment Interactions

The significant and trend G x E interactions that were detected were restricted to parental education as the environmental variable. The pre- and perinatal environmental

risk factors did not show any evidence of G x E interactions with the genetic loci under investigation. I had hypothesized that diathesis-stress G x E interactions would be detected based on previous G x E findings in ADHD with bioenvironmental risk factors. However, the ADHD G x E studies were conducted with specific alleles of candidate genes for ADHD. The results suggest that G x E interactions with bioenvironmental risk factors may be specific to certain mechanisms of action of specific genes.

Although three home environmental variables were tested in the analyses, neither books in the home nor child print exposure showed evidence of G x E interactions. In the bioecological model, Bronfenbrenner and Ceci (1994) draw a distinction between proximal environmental processes and the broader environmental context, which is referred to here as the distal environment. They specify that proximal processes are more likely to drive G x E interactions because they are closer to the mechanisms through which environments can impact developmental outcomes (Bronfenbrenner & Ceci, 1994). In contrast, distal environments are further removed from specific mechanistic explanations and provide the context in which the proximal processes occur. In these analyses, books in the home and child print exposure could be considered more proximal environmental measures because they capture more mechanistic aspects of the environment, such as access to books. Parent education could be considered a more distal environment. These results contrast with the predictions of the bioecological model, but they are not entirely unexpected. Although the mechanisms through which parent education may influence child reading are relatively unspecified, the fact that there are various mechanisms at work simultaneously (e.g., material resources, investment in

education, interest in literacy) (Smith et al., 1997) suggests that distal variables may be more powerful predictors. As a result, a logical progression for G x E studies would be to identify distal variables that reliably enter into G x E interactions. Then, follow-up studies could identify potential mechanisms and measure the relevant proximal environments that may account for the G x E interaction. These interactions with parent education must first be replicated before further mechanistic explanations should be explored. However, some of the variables measured here, albeit in small subsamples of the entire sample, may be candidate mechanisms, including family educational values, shared reading, family income, and engagement in literacy activities.

The most surprising aspect of these G x E interaction results was their directionality. I predicted bioecological interactions based on previous behavioral genetic G x E results in the same twin sample (Friend et al., 2008) and molecular genetic G x E results in a related disorder (SSD) using similar methods, genetic loci, environmental measures, and phenotypes (McGrath et al., 2007). Thus, the inconsistencies between these results must be further explored. One parsimonious explanation that cannot be entirely ruled out is that diathesis-stress G x E interactions were falsely detected because of G-E correlations (Purcell, 2002). Follow-up analyses indicated that this explanation was unlikely because the results remained robust despite statistical controls for G-E correlation. In what follows, I will first discuss the contrasts between the current results and the behavioral genetic results and then move on to the contrasting molecular genetic results.

As discussed, previous behavioral genetic analyses in the CLDRC twin sample found bioecological G x E interactions with parent education (Friend et al., 2008). Because only a subsample of the CLDRC twin sample received molecular genotyping, I explored the possibility that our diathesis-stress G x E interactions were the result of random fluctuations in the subsamples that were important for G x E directionality. However, subsample differences were of small magnitude and behavioral genetic analyses in the two subsamples showed fairly consistent evidence for bioecological G x E interactions. Theoretically, the molecular genetic G x E analyses and the behavioral genetic G x E analyses are not necessarily mutually exclusive. Behavior genetic analyses capture all of the genetic influences on RD while molecular genetic analyses focus on specific genetic loci. Thus, the molecular genetic analyses capture only a subset of the genetic variance that is considered in the behavioral genetic analyses. As a result, the behavioral genetic results could conflict with individual molecular genetic findings. However, if all of the genetic loci for RD were known (even those with very small effect sizes which are difficult to detect in molecular genetic analyses), and each of these loci were tested for G x E interactions, one would expect the behavioral genetic results to represent a summation of the molecular genetic G x E findings. At present, I cannot presume to know the locations of all of the QTLs for reading in order to directly compare the behavioral genetic and molecular genetic G x E findings. Nevertheless, it is important to note that the behavioral genetic findings could mask important complexities at the molecular genetic level, including opposing directions for G x E interactions. The design of this study as a sib-pair linkage study within a twin design highlights the

strengths of being able to compare behavioral genetic and molecular genetic findings. This design may prove increasingly informative as molecular genetic research begins to interface with the behavioral genetic tradition (Kendler, 2005). This design could answer important questions, such as whether the identified susceptibility genes for a trait can account for the observed heritability and whether behavioral genetic G x E results can be attributed to specific genetic loci.

We turn now to comparisons between the molecular genetic G x E findings in an SSD sample (McGrath et al., 2007) and the current findings. As described, the methods, phenotypes, environmental measures, and genetic loci were similar in these two studies, but bioecological G x E interactions were detected in the SSD sample and diathesis-stress G x E interactions were detected in this RD sample. Although there were notable similarities between these two studies, there were also important differences which may account for the different directionality of the G x E findings. The following explanations will be explored: (1) genetic regions of interest, (2) sample characteristics (including age, disorder, and comorbidity), and (3) environmental range. Before discussing these explanations, it is important to note that the results of McGrath et al. (2007) have not yet been replicated in an independent sample. Due to the small sample size and the exploratory nature of the analyses, the results were necessarily preliminary. One important aim of the current project was to attempt to replicate the results in a larger sample. In the discussion that follows, I will examine the results of the replication attempt more closely.

The most parsimonious explanation for the differing results is that the two studies identified G x E interactions at different genetic markers. The current study identified diathesis-stress interactions at chromosome 1 and chromosome 3 markers, whereas the SSD study identified bioecological interactions at chromosome 6 and chromosome 15 markers. The current study included markers at chromosome 6 and chromosome 15, but G x E interactions were not detected at these loci. In the SSD study, the interactions at chromosome 6 were with parent literacy and maternal education in predicting PA and RN. In the current study, I used parental education instead of maternal education, but this variable did not show interactions with chromosome 6 markers in predicting PA or RN, so I consider these results a non-replication of the previous chromosome 6 G x E findings. In the SSD study, there was also one interaction at chromosome 15 with shared reading predicting vocabulary. In the current study, I focused on phenotypes that were central to RD so I did not include a vocabulary measure. Additionally, shared reading did not show a main effect on the reading phenotypes so it was not included in the G x E interaction analyses. As a result, this study did not address the issue of replication for this G x E finding.

Although the chromosome 6 findings from the SSD study did not replicate in the current sample, it is important to consider possible explanations for the non-replication as well as possible explanations for the unpredicted directionality of the G x E interactions that were detected in the current study. The first consideration is that one sample was recruited for SSD and the other sample was recruited for RD. Although comorbidity rates between the two disorders are high (25-30%) (Gallagher, Frith, & Snowling, 2000a;

Pennington & Lefly, 2001; Scarborough, 1990b), the sample characteristics may influence the direction of the interaction. In fact, there is a precedent for contrasting G x E interactions with psychosocial risk factors in RD and ADHD, which are comorbid at similar rates as RD and SSD (Friend et al., 2008; Kremen et al., 2005; Lasky-Su et al., 2007; Laucht et al., 2007; Retz et al., 2008; Waldman, 2007).

The comorbidity rates of RD and SSD with ADHD may be an important consideration given the diathesis-stress G x E interactions that have been found for ADHD with parent education. Rates of comorbidity with ADHD are higher in RD samples than in SSD samples (McGrath et al., 2008). Children with SSD are at low risk for ADHD (13%) unless they have comorbid language difficulties (39%) (McGrath et al., 2008). The SSD sample from the McGrath et al. (2007) study had low rates of comorbid language problems so the rates of ADHD were lower in the SSD sample than in the RD sample. Because of the important potential confounding effect of ADHD in the current results, I attempted to statistically control for ADHD. The diathesis-stress G x E interactions were generally robust to these statistical controls, but these controls were limited because only a subsample of the study population had information about comorbid ADHD. Nevertheless, the results suggest that ADHD was not the determining factor in the diathesis-stress G x E interactions in the RD sample, although future research will be needed to explore this hypothesis more comprehensively. Importantly, future genetic studies of RD should collect information about comorbid ADHD so that etiological influences that are shared and distinct between these two comorbid disorders can continue to be explored.

Another important distinction between the SSD and RD samples is the age of the samples. The SSD sample was much younger (M = 5.7, SD = 0.6, range = 5-7 years) than the current RD sample (M= 10.4, SD= 2.2, range= 8-18 years). This age discrepancy raises the possibility that the form of G x E interactions may change over development. In considering this hypothesis, it is important to keep in mind that the behavioral genetic results continue to show bioecological G x E interactions in the older sample. However, behavioral genetic studies of G x E interactions have not yet been conducted in longitudinal samples. Thus, even though the G x E interaction is in the bioecological direction in the older sample, it is not clear if the effect size of this interaction may be changing across development, indicating changing molecular mechanisms underlying the behavioral genetic findings. Developmental changes in G x E interactions would not be too surprising as there is evidence for changing heritability estimates for RD across development, with RD phenotypes becoming more heritable over time (Olson, Byrne, & Samuelsson, in press; Samuelsson et al., 2008; Samuelsson et al., 2007). This change in heritability could represent changes in G x E interactions. In the classic twin design, when G x E interactions are not modeled, interactions between genetic effects and shared environmental effects inflate the heritability estimate (Plomin et al., 1977; Purcell, 2002). Thus, changes in heritability could represent developmental change in G x E interactions.

One explanation for the finding that heritability of reading phenotypes increases across development is that environmental variance accounts for more of the variance in children's reading at younger ages, but once the children reach school-age, school

exposure has a homogenizing environmental effect so that genetic differences account for more of the variance (Olson et al., in press; Samuelsson et al., 2008; Samuelsson et al., 2007). This explanation rests on the relative variance in genetic and environmental effects at different ages. However, a similar explanation can be adapted for developmental changes in G x E interactions. At younger ages, exposure to literacy activities is most important, so genetic differences are only evident in those environments which provide exposure. In environments that do not provide exposure, genetic differences are masked because these children have not yet had the opportunity to learn the skill. This pattern would be consistent with a bioecological G x E interaction as I found in our younger SSD sample. As children progress in school, the impact of poor home environments may begin to have an accumulated effect which differentially impacts children with genetic risk factors, a diathesis-stress interaction. Although this explanation could explain developmental changes in G x E interactions at specific risk alleles, this account would not fit with the overall behavioral genetic findings in older samples. Nevertheless, it is important for theoretical models of G x E interactions to begin to adopt developmental perspectives.

A final consideration is the environmental range of the two samples. One limitation of the SSD sample was the relatively high SES of the participants. The mean parental education level was 15.9 (SD = 2.6). A question left open by this previous study was whether different forms of interaction would be detected if lower SES was represented; perhaps diathesis-stress interactions would be detected when impoverished environmental circumstances were represented (McGrath et al., 2007). This RD sample

was population-based and so slightly more representative of the metro Denver area, but the SES of the participants was still quite high, consistent with the demographics of the area. Mean parental education was 14.9 (SD = 2.2). The comparable demographics of the samples make it unlikely that environmental range could explain the different forms of interaction obtained in the two samples. However, environmental range should continue to be a consideration in G x E model development.

In summary, there are several possible explanations for the unpredicted directionality of the G x E interactions that were obtained with parent education at chromosome 1 predicting PA and chromosome 3 predicting PD. Although the results can be reconciled with previous behavioral genetic and molecular genetic findings, the unpredicted direction of the interactions warrants rigorous replication of these results before additional interpretations can be made.

Limitations

The primary limitations of this study are related to sample size, genetic methodology, and robustness of the results. Although the sample size in this study was comparable to previous linkage studies (for a review see Pennington et al., in press), G x E interactions are notoriously difficult to detect and replicate (Rutter, 2006). The sample size was further limited in specific analyses because certain measures were introduced after the beginning of the study, such as the ADHD measures and the parental RHQ. These two measures were important for exploring alternative explanations for the diathesis-stress G x E interactions, including comorbidity and G-E correlation. As a

result, I could not comprehensively address these competing explanations although statistical controls in the existing samples suggested that these alternatives were unlikely.

Secondly, I used a linkage framework to conduct the G x E analyses. Linkage studies can only identify a genetic neighborhood of interest in which one or more QTLs for RD are likely to reside. Because researchers have not yet identified replicable causal variants in the identified susceptibility genes for RD, these linkage methods remain the best methodology available to test for G x E interactions. However, there has been much criticism of linkage studies because very few linkage peaks have led to replicable candidate genes (Bourgain, Genin, Cox, & Clerget-Darpoux, 2007). As genome-wide association studies are completed in various disorders, the lack of correspondence between linkage and association signals has been discouraging for many medical disorders (Bourgain et al., 2007). Critics of linkage studies suggest that the linkage signals are mostly composed of noise and so G x E interactions in a linkage framework are unlikely to be meaningful (Bourgain et al., 2007). At this point, it is unclear if RD may be an exception to the disappointing linkage results in other disorders. RD may be one of the few success stories in which linkage peaks have led to the discovery of replicable candidate genes (Fisher & Francks, 2006; McGrath et al., 2006). However, until a genome-wide association study is conducted in RD, it will be unclear if the candidate genes identified through the linkage signals will overlap with the strongest association signals.

The use of a linkage framework also limits the ability to test for G-E correlations directly. As discussed, these correlations are an important statistical confound in tests of

G x E interaction. Because linkage studies only identify a genetic neighborhood that is shared between concordant siblings and unshared between discordant siblings, it is difficult to determine which individuals possess a particular risk allele. In an association framework, G-E correlations could be tested directly by examining whether parental risk alleles were associated with environmental risk factors (passive G-E correlations) and whether child risk alleles where associated with environmental risk factors (active and evocative G-E correlations). Because I could not test for G-E correlations directly, I used self-report measures of parental reading history to index parental genetic risk for RD and control for passive G-E correlations. This method is obviously imprecise, but more sophisticated controls for G-E correlation will await replicable association results with specific RD risk alleles.

A final limitation relates to the robustness of the results. For example, the G x E interactions were only detected at the trend-level linkage peaks. I conducted G x E interactions analyses at these trend linkage peaks because G x E interactions can obscure the linkage signal when the interaction is not modeled. One of these interactions reached significance (p <.01) and one was itself a trend (p <.05). When several phenotypes were identified within a single linkage peak, there was not much correspondence between the G x E interactions with the phenotypes. For example, on chromosome 1, the Wrec, PD and PA phenotypes all showed linkage, but only the PA phenotype showed evidence of a G x E interaction. The correlations between these phenotypes ranged from r = .7 - .8. Because there is no theoretical reason to expect that parental education would show an interaction in one of these phenotypes but not the other, it is difficult to interpret the

meaning of the differential results. Finally, the unpredicted direction of the interaction lends additional caution to the interpretation. Despite these concerns, the converging evidence for the same G x E interactions across two different linkage methods and the robustness of the results to several different statistical controls is encouraging. Taken together, I consider the results preliminary, but worthy of further exploration.

Future directions

As previously mentioned, an important future direction will be to test for G x E interactions in an association framework where the candidate gene is identified and where G-E correlations can be tested directly.

The precision in phenotypes that was necessary to detect linkage in this sample suggests that latent trait models that model shared and error variance will be increasingly important in genetic analyses. Within the linkage framework, multivariate linkage analysis has been successfully performed in RD and SLI (Marlow et al., 2003; Monaco, 2007), but these maximum likelihood models are not optimal for selected samples without appropriate corrections (Hawke et al., 2008). An important future direction is for these multivariate models to be extended to incorporate G x E interactions.

As these modeling developments indicate, there has been increasing interest in phenotypic precision and endophenotypes in the genetics literature (Gottesman & Gould, 2003). The G x E interaction literature would benefit from a similar emphasis on environmental measurement and specification (Moffitt et al., 2006). Similar to the phenotypes in RD, psychosocial environmental measures are often multifaceted and

correlated. Thus, models of G x E interactions should begin to incorporate multivariate definitions of the environment.

The other primary direction for future research concerns the development of G x E interaction models in developmental disorders. G x E interaction research is still in its infancy and so the models are currently under-specified for making predictions about the direction of expected interactions. Exploration of the current results led to 5 salient dimensions that will need to be incorporated into existing G x E models: disorder of interest, comorbidity, nature of the environmental variable, environmental range, and developmental trajectory. At present, the role that each of these dimensions plays in determining the mechanisms of G x E interaction is unclear. G x E model development will be greatly advanced by research that compares interactions across disorders and considers comorbidities, different environmental variables (e.g., psychosocial versus bioenvironmental), and the full range of environmental variance. Longitudinal samples with genetically sensitive designs will be crucial for understanding the changing nature of G x E interactions across development.

Overall, as psychiatric and molecular genetics continues to flourish in this post-genomic era, G x E interaction research is likely to make substantial contributions to developmental theory and the understanding of complex developmental disorders.

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Appendix – Supplementary Mailing

Home Questionnaire

Name of person completing this questionnaire: _____
Relationship to the twins (e.g., mother, father):_____
Date: _____

Family Priorities

There are differences in opinion and differences in the research literature regarding the benefits of certain activities for children under the age of 5. We are interested in what activities you think families should do with young children during their leisure time. What are the top 3 activities that you feel are important to do with children under 5?

1. _____
2. _____
3. _____

Why do you think these are the most important for young children?

_____

_____

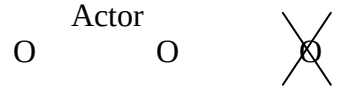
_____

All families have time constraints that sometimes prevent them from doing the activities that they would like with their young children. For your family, what were the 3 things that you (one or both parents) did together the most with your twins?

1. _____
2. _____
3. _____

The next set of questions will all follow the same pattern. We will ask which person (child, parent, teacher) you feel is more responsible for something.

Here is a pretend example: Who is more responsible for making a good movie? We think an actor is mostly responsible but the director is a little responsible for making a good movie, so we marked the bubble as below.



Who do you think is more responsible for teaching a child new words, a teacher or a parent?

Teacher Parent

Who do you think is more responsible for making sure a child is successful in school, a teacher or a parent?

Teacher Parent

Who do you think is more responsible for making sure a child is successful in school, a child or a parent?

Child Parent

Who do you think is more responsible for making sure a child is successful in school, a teacher or a child?

Teacher Child

Who do you think is more responsible for a child learning to read, a teacher or a parent

Teacher Parent

Who do you think is more responsible for a child learning to read, a child or a parent?

Child Parent

Who do you think is more responsible for a child learning to read, a teacher or a child?

Teacher Child

Over time, people's attitudes sometimes change for various reasons, such as personal experiences or shifts in scientific knowledge or cultural values. Would you have answered these questions differently when your twins were 3-4 years old?

a. yes b. no

If yes, please describe how your attitudes have changed:

_____
_____
_____
_____

Current Home Environment

Please fill out the following questions regarding your (parent) current home activities.

1) What is your (parent) current attitude toward reading?

Very positive Very Negative

0 _____1 _____2_____3_____4

2) How much reading do you (parent) do for pleasure?

A great deal Some None

0 _____1 _____2_____3_____4

3) How many books do you (parent) read each **month?**

a. None
b. 1-2
c. 3-6
d. 7-10
e. Over 10

4) How many magazines do you (parent) read each **month**?

a. None
b. 1-2
c. 3-6
d. 7-10
e. Over 10

5) Estimate how many books you presently have in your home.

a. 0 - 50
b. 51 - 100
c. 101 - 200
d. 201 - 500
e. Over 500

6) How often do people in your family buy books for themselves (including parents buying books for the children)?

a. Hardly ever
b. Less than 5 times per year
c. 5-10 times per year
d. 10-15 times per year
e. More than 15 times per year

7) How often do people in your family buy books to give as presents to others?

a. Hardly ever
b. Less than 5 times per year
c. 5-10 times per year
d. 10-15 times per year
e. More than 15 times per year

8) How often do people in your family check books out of the library?

a. Hardly ever
b. Several times a year
c. Once or twice a month
d. Several times a month
e. Every week

9) Have you ever (or do you presently) read to your children?

a. Never
b. Rarely
c. Sometimes
d. Regularly, I love to

Early Home Literacy Practices
Please fill out the following questions regarding reading that you did with your twins at <u>home</u> early in their development.

Many of the following questions will ask about your activities with the twins when they were 3-4 years old. Please try to remember the events of your life when your twins were 3-4 years old. Think back to the school, daycare, and playgrounds that they attended and the neighborhood and house in which you lived in order to jog your memory of that time period.

For each of these questions, we would like you to answer the question for both twins that participated in our research study. Please fill in their first names where indicated and provide the answer that is relevant to that child.

1) At what age did you or another family member/caretaker begin to read to your twins?

Twin 1 (insert name): _____
- a. 0-6 months
- b. 7-12 months
- c. 13 months to 1½ years
- d. 1½ years to 2 years
- e. later than 2 years

Please provide an exact estimate:
Age in **<u>months</u>**: _____

Twin 2 (insert name): _____
- a. 0-6 months
- b. 7-12 months
- c. 13 months to 1½ years
- d. 1½ years to 2 years
- e. later than 2 years

Please provide an exact estimate:
Age in **<u>months</u>**: _____

If the ages differed for the two twins, please describe why (e.g., one twin was more interested or enjoyed the activity more etc.)

_____
_____
_____

2) How often did you or another family member/caretaker read out loud to or with your twins when they were 3-4 years old?

Twin 1 (insert name): _____

a. Hardly ever
b. Once or twice a month
c. Once or twice a week
d. Several times a week
e. Almost daily/daily
f. Several times per day

Twin 2 (insert name): _____

a. Hardly ever
b. Once or twice a month
c. Once or twice a week
d. Several times a week
e. Almost daily/daily
f. Several times per day

If the answers differed for the twins, please describe why (e.g., one twin always requested to be read to or one twin was more interested in other activities etc.):

_____
_____
_____
_____

3) How often did you or another family member/caretaker go to the library with your twins when they were 3-4 years old?

Twin 1 (insert name): _____

a. Hardly ever
b. Several times a year
c. Once or twice a month
d. Several times a month
e. Every week

Twin 2 (insert name): _____

a. Hardly ever
b. Several times a year
c. Once or twice a month
d. Several times a month
e. Every week

If the answers differed for the twins, please describe why:

_____
_____
_____

4) Approximately how many picture books did you have in your home when the twins were 3-4 years old?

a. 0 – 2
b. 3 – 10
c. 11 – 20
d. 21 – 40
e. more than 40

Out-of-Home Care

The following questions refer to your twins' out-of-home care when they were 3-4 years old.

1) When your twins were 3-4 years old, did they attend any out-of-home care before starting school? (e.g., preschool, daycare etc.)

Twin 1 (insert name): _____
a. yes b. no

If yes, what type _____

At what age **in months** did your twin begin attending this out-of-home care?
_____

For how many years did your twin attend?
_____

Was it educationally focused?
(e.g., learning letters, learning numbers)
a. yes b. no

Twin 2 (insert name): _____
a. yes b. no

If yes, what type _____

At what age **in months** did your twin begin attending this out-of-home care?
_____

For how many years did your twin attend?
_____

Was it educationally focused?
(e.g., learning letters, learning numbers)
a. yes b. no

If the out-of-home care arrangements differed for the twins, please explain why (e.g., only one twin could be accepted into the daycare/preschool etc.)

_____
_____
_____

If your twins had multiple arrangements or you would like to add additional comments, please add them here:_____
_____
_____

Family Income

We are collecting the following information to describe the sample of children in this study so that other researchers will understand the population of children and families for which the collected data are relevant. This information will be kept completely confidential. As always, please feel free to skip any questions that you do not feel comfortable answering.

What was your family's estimated annual household income when the twins were 3-4 years old?

_____ <$10,000	_____ $40,000 - $49,999	_____ $80,000 - $89,999
_____ $10,000 – $19,999	_____ $50,000 - $59,999	_____ $90,000 - $99,99
_____ $20,000 – $29,999	_____ $60,000 - $69,999	_____ >$100,000
_____ $30,000 - $39,999	_____ $70,000 - $79,999	

At that time, how many people were supported by that income? _____