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Genetic Moderation of Early Child-Care Effects on Social Functioning Across Childhood: A Developmental Analysis

Jay Belsky
University of California, Davis and King Abdulaziz University, and Birkbeck University of London

Michael Pluess
Institute of Psychiatry, King's College of London

Data from 508 Caucasian children in the NICHD Study of Early Child Care and Youth Development shows that the DRD4 (but not 5-HTTLPR) polymorphism moderates the effect of child-care quality (but not quantity or type) on caregiver-reported externalizing problems at 54 months and in kindergarten and teacher-reported social skills at kindergarten and first grade—but not thereafter. Only children carrying the 7-repeat allele proved susceptible to quality-of-care effects. The behavior–problem interactions proved more consistent with diathesis-stress than differential-susceptibility thinking, whereas the reverse was true of the social-skills' results. Finally, the discerned Gene × Environment interactions did not account for previously reported parallel ones involving difficult temperament in infancy.

Debate has long characterized discussion of the effects of child care on children's development (Fox & Fein, 1990; Karen, 1998), although this is clearly more true regarding adverse effects of lots of time spent in child care (Belsky, 1986, 1988, 1990; Clarke-Stewart, 1989; Phillips, McCartney, Scarr, & Howes, 1987; Vandell, Belsky, Burchinal, Steinberg, & Vandergrift, 2010), perhaps particularly centers (Belsky, Vandell, Burchinal, Clarke-Stewart, McCartney, & Owen, 2007), than with respect to quality of care. After all, it is not only widely believed that quality of child care is an important determinant of children's functioning, but there is long-standing evidence consistent with this claim (e.g., Howes, 1988; Peisner-Feinberg & Burchinal, 1997; Vandell, Henderson, & Wilson, 1988).

Important to appreciate, however, is that inconsistency exists within the research literature even with respect to the effects of child-care quality. Perhaps most notable in this regard is the general failure of careful measurements of how attentive, responsive, and stimulating caregivers were to children enrolled in the large-scale NICHD Study of Early Child Care and Youth Development (SECCYD) to predict children's social adjustment after age 3 and before age 15, even as it consistently predicted children's cognitive-linguistic functioning and academic achievement during this developmental period (Belsky et al., 2007; NICHD ECCRN, 2003; Vandell et al., 2010).

For the past decade or so, developmentalists studying the effects of diverse environmental experiences and exposures have become ever more aware that individuals may differ in whether and how they are affected by their developmental experiences and, most importantly for the research reported herein, that this may be a function of genetics (Caspi & Moffitt, 2006). Although some have questioned the replicability of particular Gene × Environment (G×E) interactions that have appeared in the published literature (Duncan & Keller, 2011; Risch et al., 2009), it would certainly seem mistaken to throw the genetic-moderation-of-environmental-influences baby out with the bathwater (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Karg,

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Correspondence concerning this article should be addressed to Jay Belsky, Human and Community Development, University of California, Davis, One Shields Avenue, Hart Hall, Davis, CA 95616. Electronic mail may be sent to jbelsky@ucdavis.edu.

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Burmeister, Shedden, & Sen, 2011; Rutter, Thapar, & Pickles, 2009; Uher & McGuffin, 2010). Indeed, here we take advantage of data from the NICHD SECCYD to examine for the first time whether two particular genetic factors might moderate effects of quality, quantity, and type of child care on children's teacher-rated externalizing behavior problems and social skills. In so doing, we are positioned to shed some light on at least one reason why child-care effects have been subject to so much debate. After all, if at least some child-care effects prove to be genetically moderated, varying across studies due to the genetic makeup of samples, this could account for some of the inconsistency in the literature. To be appreciated, however, is that because the sample studied here is limited in size, is not epidemiological in character, and we do not have a second sample on which to replicate any detected G×E effects, the research presented herein represents a proof-of-concept effort. While it can shed light on whether child-care effects could vary as a function of children's genotype, it is not positioned to provide compelling evidence that they certainly do. Additional research will be required before such could be claimed.

The Moderation of Child-Care Effects

As it turns out, there is evidence that some child-care effects vary as a function of children's characteristics of individuality. Pluess and Belsky (Belsky & Pluess, 2012; Pluess & Belsky, 2009, 2010) found, upon further analyzing NICHD SECCYD data, that early temperament played a moderational role with respect to effects of quality of care, and it did so in ways consistent with some other work evaluating this possibility (Crockenberg, 2003; Phillips, Fox, & Gunnar, 2011). More specifically, children who were rated as more difficult to care for in their first 6 months of life, including being more negatively emotional, proved to be more affected by quality—but not quantity or type—of care than did other children, at least with respect to externalizing problems and social skills. Just as intriguing was the fact that such children were affected in a for-better-*and*-for-worse manner (Belsky, Bakermans-Kranenburg, et al., 2007). That is, children with histories of difficult temperament in infancy scored higher on behavior problems than all other children across the preschool, middle-childhood, and adolescent years if they had experienced low-quality care, yet lower on behavior problems than others if they had experienced high-quality care (Belsky & Pluess, 2012; Pluess & Belsky, 2009, 2010).

Models of Environmental Action

Such moderational effects of temperament vis-à-vis child-care experiences were particularly interesting in that they proved more consistent with the differential-susceptibility hypothesis (Belsky, Bakermans-Kranenburg, et al., 2007; Belsky & Pluess, 2009; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011) than the prevailing diathesis-stress model of environmental action (Zuckerman, 1999). The long-standing and highly influential diathesis-stress framework exclusively addresses the issue of who is most susceptible to the *negative* effects of *adverse* environmental experiences and exposures (e.g., poverty, harsh parenting), essentially presuming that some are more *vulnerable* than others as a result of a personal characteristic (e.g., difficult temperament, risk allele). It is this conceptual framework that informed the first G×E interaction research examining the effects of maltreatment on antisocial behavior (Caspi et al., 2002) and of life event stress on depression (Caspi et al., 2003).

Notably, the diathesis-stress paradigm does not in any way address differential response to positive, supportive, or enriching environmental experiences and exposures (e.g., high-quality child care, sensitive parenting), and this is where it differs from the differential-susceptibility perspective. Rather than regarding some individuals as exclusively more vulnerable than others to adversity due to some endogenous factor, the differential-susceptibility model of environmental action presumes that those most likely to be negatively affected by some adverse environmental condition are also most likely to *benefit* from a *supportive* one. Thus, whereas diathesis-stress thinking regards some as more vulnerable to adversity, differential-susceptibility thinking stipulates that those most susceptible to negative environmental influences are simultaneously most susceptible to positive ones as well. That is, they are not so much vulnerable as malleable or developmentally plastic—for-better-*and*-for-worse. Intriguingly, a good deal of G×E interaction work, the focus of the current report, provides evidence consistent with differential-susceptibility theorizing (Belsky et al., 2009; Belsky & Pluess, 2009; Ellis et al., 2011).

G×E Interaction

In the current report, we extend examination of the potential moderating effect of child attributes with respect to the potential influence of day care

(i.e., quality, quantity, and type) on teacher-rated social adjustment by focusing on children's genetic makeup. In particular, in the G×E analyses reported herein, we explore the prospect that the serotonin transporter gene, 5-HTTLPR, or the DRD4 dopamine-receptor gene moderate the effect of child-care experience on teacher-rated externalizing problem behavior and social skills, perhaps accounting for why quality of care in particular did not predict—as a main effect—social adjustment from just before school entry to the last year of elementary school in the NICHD SECCYD (Belsky et al., 2007; Belsky, Bakermans-Kranenburg, et al., 2007). We restrict our analyses to these two polymorphisms, despite the fact that a range of VNTRs and SNPs have been genotyped on the SECCYD cohort, because they have proven most often in G×E research to function as "plasticity genes" in line with differential-susceptibility rather than just "risk alleles" consistent with diathesis-stress models of environmental action (Belsky & Pluess, 2009).

There are several additional reasons to focus on the DRD4 polymorphism in this study of the effects of quality, quantity, and type of care on externalizing problem behavior and social skills. First, DRD4 has been linked with aggression (and antisocial behavior) in children and adolescents (Beaver et al., 2007; Oades et al., 2008; Schmidt, Fox, Rubin, Hu, & Hamer, 2002), a core component of externalizing problems that is one of the two developmental outcomes that are the focus of this inquiry and that has been the source of so much controversy with respect to day-care effects (Crockenberg, 2003; Fox, 1990; Langlois & Liben, 2003; Maccoby & Lewis, 2003). Second, the DRD4 polymorphism has been related to negative emotionality in infancy (Holmboe, Nemoda, Fearon, Sasvari-Szekely, & Johnson, 2011), thereby raising the prospect that the repeatedly chronicled and aforementioned Temperament × Child Care quality interaction discerned by Pluess and Belsky (Belsky & Pluess, 2012; Pluess & Belsky, 2009, 2010) could be the result of a G×E interaction involving DRD4; we evaluate this very possibility in the final analysis presented in this report.

A third and perhaps more important reason for focusing on the DRD4 polymorphism in this inquiry is that Dutch investigators have found that it moderates the effect of quality of parenting on externalizing behavior problems in both observational research (Bakermans-Kranenburg & van IJzendoorn, 2006) and intervention work (Bakermans-Kranenburg, van IJzendoorn, Pijlman, Mesman, & Juffer, 2008). Specifically, children carrying the 7-repeat allele proved more susceptible to parenting effects than did children not carrying this version of the gene, and they did so in a manner consistent with differential susceptibility. Because the measurements of quality of child care in the NICHD study tap much the same caregiving behavior of child-care providers as did the measures of parenting examined in these Dutch studies, it certainly is conceivable that this same polymorphism might moderate the effects of child-care quality—and perhaps quantity and type of care as well—on externalizing behavior problems and social skills. This possibility would seem further buttressed by the fact that a 10-study meta-analysis revealed that variation in genes related to dopamine signaling in the brain influence children's sensitivity to both sensitive or responsive *and* harsh or unresponsive parenting (Bakermans-Kranenburg & van IJzendoorn, 2011).

Reasons for focusing on the serotonin transporter gene, 5-HTTPLR, as a possible moderator of child-care effects in this report are much the same as those highlighted in the case of DRD4. It, too, has been specifically implicated in moderating effects of parenting on child development (e.g., Barry, Kochanska, & Philibert, 2008) and has been found to be related to negative emotionality (Auerbach et al., 1999), even moderating the effect of prenatal stress on negative emotionality in infancy (Pluess et al., 2011). In fact, a recent meta-analysis of G×E studies involving children under 18 years of age reveals 5-HTTLPR to operate as a "plasticity gene"—with carriers of short alleles disproportionately benefiting from supportive environmental conditions while also being most adversely affected by negative contextual conditions more than others (van IJzendoorn, Belsky, & Bakermans-Kranenburg, in press).

Current Study

In sum, in this work we address, for the first time, whether effects of day care are genetically moderated, by focusing on two particular polymorphisms, DRD4 and 5-HTTLPR, and social adjustment as measured by repeated teacher reports of externalizing behavior problems and social skills across the middle-childhood years. It should be noted that this study involves a smaller sample than used in most analyses of NICHD SECCYD data due to the fact that not all children provided DNA. Although this makes precise comparison with other NICHD SECCYD reports difficult, it is not unusual for parents to turn down the opportunity for genetic data to be collected on their children.

We further seek to determine whether any detected G×E effects prove consistent with diathesis-stress or differential-susceptibility theorizing. In addition, we endeavor to determine whether conclusions that only quantity and type of child care, not quality of care, predict social adjustment, at least during the period from just before or at time of school entry to the end of the elementary school years, need to be modified as a result of the detection of G×E interaction involving quality of care. In so doing, we extend prior work showing that at least one child attribute, early difficult temperament, moderates the effect of child-care quality on externalizing problems and social skills. Indeed, we additionally seek to determine, to our knowledge for the first time, whether a previously documented moderational effect of a feature of temperament is independent of genetic moderation that might be discerned in this inquiry. In view of the fact that previously cited research chronicles links between DRD4 and 5HTTLPR and negative emotionality, (Auerbach et al., 1999; Holmboe et al., 2011) there are certainly grounds for predicting that a G×E effect involving one or both of these polymorphisms could account for a parallel moderational effect involving difficult temperament.

Finally, given the availability of repeated measurements of our dependent variables from just before or during the kindergarten year through sixth grade, we seek to determine whether G×E effects prove to be time limited or endure. Typically, as the time between the measurement of a developmental experience and the developmental outcome it is thought to influence increases, the strength of the association between the two decreases. We can see no reason why this would not apply to a genetically moderated effect of early experience, in this case child-care experience. Thus, we not only seek to determine whether DRD4 moderates the effect of child care around the transition to school, but whether it continues to do so through the elementary school years. We do this by determining whether the child-care-related G×E interactions under investigation predict the slope—or change—in the outcomes studied from initial time of measurement just before school entry (behavior problems) or in kindergarten (social skills) to about 12 years of age, and if so, we then evaluate child-care-related G×E effects on social functioning at subsequent measurement occasions. With the exception of two very recent studies (Petersen et al., 2012; Sulik et al., 2011), virtually all G×E work to date has focused exclusively on outcomes measured at one point in time. Thus, it is impossible to determine whether detected G×E effects dissipate, strengthen, or remain unchanged over time. Unless the possibility that G×E effects change over time is evaluated, the enduring nature of any discerned G×E effect cannot be known.

Method

Participants

Families were recruited through hospital visits to mothers shortly after the birth of a child in 1991 in 10 locations in the United States. During the selected 24-hr intervals, all women giving birth (n = 8,986) were screened for eligibility. From that group, 1,364 families completed a home interview when the infant was 1 month old and became study participants. DNA (buccal cell swabs) was collected when children were 15 years old. Details of the sampling plan can be found in NICHD ECCRN (2005a).

Only children of Caucasian ethnicity were included in this study to avoid confounding effects of ethnic differences in gene frequency. Genetic data were available for 516 of the 1,097 Caucasian children. Of the 516 children with genetic data, 8 were excluded from the analysis sample due to having no outcome data, resulting in a final sample of 508 children. Children excluded, relative to those included, came from households with lower income-to-needs ratios (2.93 vs. 3.89) and with less educated (13.96 vs. 14.70 years) and more depressed (10.27 vs. 9.23) mothers, who were more likely to be single parents (20.80% vs. 9.35%) and who provided lower quality parenting (−.16 vs. .18). Excluded children were rated as having a more difficult temperament in infancy (3.21 vs. 3.13), experienced a lower proportion of center child care prior to starting school (.19% vs. .23%), had more externalizing behavior problems at every assessment from kindergarten (50.30 vs. 49.05) through sixth grade (51.05 vs. 49.32), and had less social skills at every assessment from kindergarten (102.31 vs. 104.81) through sixth grade (101.23 vs. 104.50). Characteristics of the final sample and means of all variables are displayed in Table 1.

Measures

With the exception of DRD4, 5HTTLPR, and difficult temperament, most measures described are very similar to those used in Belsky et al.'s (2007) first evaluation of longer term child-care effects in the NICHD study. Additional information about

Table 1
Sample Characteristics

Variables	*N*	*M* (*SD*)
Maternal education at 1 month [years]	508	14.70 (2.39)
Income-to-needs ratio (across 54 months)	507	3.89 (2.70)
Maternal depression (across 54 months)	508	9.23 (6.18)
Parenting quality (across 54 months)	505	0.18 (0.58)
Partner presence (across 54 months)	508	90.65 (23.16)
Child temperament at 6 months	499	3.13 (0.40)
Child-care quality (across 54 months)	464	2.82 (0.24)
Child-care hours (across 54 months)	502	25.89 (16.07)
Center child care (across 54 months)	495	0.23 (0.28)
Child gender		
Boy	251	49.4%
Girls	257	50.6%
Child DRD4		
DRD4-7R present	135	26.6%
DRD4-7R absent	373	73.4%
Child 5-HTTLPR		
l/l	143	28.1%
l/s	255	50.2%
s/s	110	21.7%
Child externalizing behavior at 54 months	354	49.75 (9.53)
Child externalizing behavior at KG	465	49.05 (8.53)
Child externalizing behavior at G1	471	49.76 (8.32)
Child externalizing behavior at G2	451	49.50 (8.26)
Child externalizing behavior at G3	471	50.40 (8.37)
Child externalizing behavior at G4	454	49.18 (8.21)
Child externalizing behavior at G5	464	49.78 (8.27)
Child externalizing behavior at G6	439	49.32 (8.91)
Child social skills at KG	458	104.81 (13.61)
Child social skills at G1	467	105.06 (13.06)
Child social skills at G2	449	106.86 (13.45)
Child social skills at G3	467	103.84 (13.41)
Child social skills at G4	453	104.16 (13.16)
Child social skills at G5	464	104.23 (13.73)
Child social skills at G6	430	104.50 (14.08)

procedures and measures are provided in *Manuals of Operation* of the study, located at http://www.nichd.nih.gov/research/supported/seccyd.cfm.

Child-Care Characteristics

Nonfamilial child care was defined as regular care by anyone other than the mother, father, or grandparents—including nannies (whether in home or out of home), family day-care providers, and centers. Three aspects of child-care experiences were measured.

Child-care quantity. Parents reported children's hours of routine nonfamilial care during phone and personal interviews conducted at 3- or 4-month intervals from ages 1–54 months, as well as the type(s) of child care used. The *hours* spent in all settings were summed for each of the 17 intervals or "epochs" and parameterized on an hour per week basis. Individual measures of level and rate of change in quantity of care were computed as the individual intercepts and slopes from an unconditional hierarchical linear models (HLM) analysis of these 17 repeated measures. Following NICHD ECCRN (2003), age was centered at the measurement midpoint, 27 months, so the estimated intercept reflected that child's hours per week at 27 months of age.

Child-care type. For each measurement epoch, each of the child's care arrangements was classified as center, child-care home (any home-based care outside the child's own home except care by grandparents), in-home care (any caregiver in the child's own home except father or grandparent), grandparent care, or father care. The proportion of epochs in which the child received care in a center for at least 10 hr per week was used to represent the type of care.

Child-care quality. Multiple observational assessments using the Observational Record of the Caregiving Environment were conducted in the primary child-care arrangement at ages 6, 15, 24, 36, and 54 months to evaluate how sensitive, responsive, stimulating, positive and nonnegative, and nonintrusive caregiving proved to be; for measurement details, see NICHD ECCRN (2002). As with quantity, individual measures of level and change in quality (i.e., slope) were estimated with an unconditional HLM analysis (NICHD ECCRN, 2003).

Covariates

Early childhood. Following Belsky et al. (2007), measures of maternal, child, and family characteristics during infancy and early childhood were collected and used as controls for possible selection bias: *child gender*, *maternal education* (years of schooling at time of child's birth), the proportion of (five measurement) epochs through 54 months in which the mother reported a *husband* or *partner* was present, family income through 54 months calculated as the mean *income-to-needs ratio*, and the mean of *maternal depressive symptoms* assessed by the Center for Epidemiological Studies Depression Scales reported by the mother at 6, 15, 24, 36, and 54 months. These early childhood covariates were included in the reported HLM analyses as time-invariant controls.

Difficult temperament. Temperament was assessed by maternal report at 6 months using an adapted

version of the Infant Temperament Questionnaire (Carey & McDevitt, 1978). Items were designed to capture approach, activity, intensity, mood, and adaptability. An overall summary of "difficultness" was calculated with higher values reflecting higher negative emotionality.

Parenting quality 6–54 months. Parenting quality was assessed by (a) maternal sensitivity in semistructured play and (b) observation at home:

1. *Maternal sensitivity*: Mother–child interactions were videotaped in semistructured 15-min observations at 6, 15, 24, 36, and 54 months, with interactional tasks enabling evaluation of age-appropriate qualities of maternal behavior. Videotapes were coded at a central location by raters blind to other information about the families. Intercoder reliability was determined by assigning two coders to 19%–20% of the tapes randomly drawn at each assessment period. Intercoder reliability was calculated as the intraclass correlation coefficient. Reliability for the composite scores used in the current report exceeded .83 at every age. At 6, 15, and 24 months, composite maternal sensitivity scores were created from the sums of three 4-point ratings (maternal sensitivity to child nondistress, intrusiveness [reversed], and positive regard). At 36 and 54 months, the maternal sensitivity composite was the sum of the three 7-point ratings of supportive presence, hostility (reversed), and respect for autonomy. Cronbach's alphas exceeded .70 at every age.
2. The *Home Observation for Measurement of the Environment* (HOME; Caldwell & Bradley, 1984) was administered during home visits at 6, 15, 36, and 54 months. The Infant or Toddler version of the inventory (IT-HOME), composed of 45 items was used across the first 3 years of life. The Early Childhood version of the inventory (EC-HOME), composed of 55 items, was used at 36 and 54 months. A centrally located system of training was used for data collectors at each age. Cronbach's alphas for the total score at each age exceeded .77.

The HOME total and maternal sensitivity ratings were standardized and averaged at each age and then across the first 54 months to create a composite score measure of early parenting quality.

Primary grades. Measures of family demographic and psychological characteristics also were obtained when children were in kindergarten and in first, third, and fifth grades. Following Belsky et al. (2007), the following were included as time-varying covariates in the HLM analyses: *presence of a husband* or *partner* in the household, *income-to-needs ratio*, *maternal depressive symptoms*, and *parenting quality*. Composite *parenting quality* scores for the primary grades were created similar to parenting quality for 6–54 months (see above) by averaging standardized ratings of observed maternal sensitivity and standardized ratings of observed home environmental quality (HOME; Caldwell & Bradley, 1984), which were assessed at 54 months and first (only maternal sensitivity), third, and fifth grades.

In addition to family-related covariates, *classroom quality* and *after-school experience* during the primary grades were included as controls. Children's classroom experiences were measured using the *classroom observation system for first grade* (Allhusen et al., 2004), *for third grade* (NICHD ECCRN, 2005b), and *for fifth grade* (NICHD ECCRN, 2004). These observations focused on the classroom as well as the specific study child and his or her classroom experiences. Three 7-point global ratings of the classroom environment were made at the end of two (first grade) or eight (third and fifth grades) 44-min observation cycles: overcontrol by teacher, teacher's emotional detachment, teacher's sensitivity to student needs.

Regarding after-school experience, mothers were interviewed by telephone in the fall and spring of kindergarten and first, third, and fifth grades about the study children's out-of-school care. Following Belsky et al. (2007), hours of nonparental out-of-school care arrangements (here named *after-school hours*) were obtained for each school year from the average across the spring and fall reports of the total hours mothers reported across all nonparental out-of-school care arrangements.

Child Outcomes

Externalizing behavior problems. Teachers reported on children's *externalizing behavior problems* (e.g., "hits others," "disobedient at school," "argues a lot") repeatedly—at 54 months, kindergarten, and annually in first, second, third, fourth, fifth, and sixth grades—using the Child Behavior Checklist Teacher Report Form (Achenbach, 1991). Raw scores were converted into standard *T* scores, based on normative data for children of the same age.

Social skills. Teachers reported on children's *social competence and social skills* (e.g., "makes friends easily," "controls temper when arguing with other children," "asks permission before using someone else's property") repeatedly using the Social Skills

Questionnaire from the Social Skills Rating System (Gresham & Elliott, 1990)—beginning at kindergarten and annually thereafter in first, second, third, fourth, fifth, and sixth grades. For purposes of this report, raw total scores were converted into standardized scores, based on normative data for children of the same age.

Genetic Analyses

DNA was extracted from buccal cell swabs (Freeman et al., 2003). The majority of samples were genotyped twice for both DRD4 (n = 438, 86.2%) and 5-HTTLPR (n = 465, 91.5%) to evaluate the reliability of genotyping. If there was a discrepancy between the two assessments, genotyping was repeated until the same result was found twice. We defaulted to the original genotype, however, if a sample could not be genotyped a second time or if we were unable to identify a single genotype for a given sample.

DRD4 was identified using a modified assay based on methods developed by Sander et al. (1997) and modified by Anchordoquy, McGeary, Liu, Krauter, and Smolen (2003): 1× Taq Gold Buffer, 2.25 mM final concentration of $MgCl_2$, 10% DMSO, 0.2 mM dNTPs, 0.1 mM deazo GTP, 0.75 μM primers, 40 ng of DNA, and 1 U of Taq Gold (Applied Biosystems, Foster City, CA) in a volume of 12 μl. The primer sequences were: 5′–6-FAM-GCGAC TACGTGGTCTACTCG-3′ and reverse, 5′-AGGACC CTCATGGCCTTG-3′. The amplification procedure was as described by Anchordoquy et al. (2003). One microliter was removed and placed in a 96-well plate and 10 μl of formamide containing LIZ-500 standard (Applied Biosystems). The plate was run using a Fragment Analysis protocol in the 3730XL DNA Analyzer (Applied Biosystems). Fragments were analyzed using Genemapper software (Applied Biosystems) with PCR products of (in bp): 379, 427, 475 (43), 523, 571, 619 (73), 667, 715, 763, and 811. DRD4 was coded as individuals carrying one or more 7-repeat allele versus all others. Agreement between first and second genotyping was 86.5% (κ = .63, p < .001). For the 13.5% where the two rounds of genotyping proved discrepant, a third round was conducted that determined what genotype these discrepant cases would be assigned. The DRD4 7-repeat allele was present in 26.6% of the final sample.

5-HTTLPR was identified using a modified assay based on the method of Lesch et al. (1996) and Anchordoquy et al. (2003): 1× Taq Gold Buffer, 1.8 mM final concentration of MgCl2, 10% DMSO, 0.2 mM dNTPs, 0.1 mM deazo GTP, 0.6 μM primers, 40 ng of DNA, and 1 U of Taq Gold (Applied Biosystems) in a volume of 15 μl. The primer sequences were: forward, 5′-VIC-GGCGT TGCCGCTCTGAATGC-3′ and reverse, 5′-GAGGG ACTGAGCTGGACAACCAC-3′. The same amplification protocol was used as for DRD4 (see above). Fragments were analyzed using Genemapper software (Applied Biosystems) with PCR products of 484 or 528 bp. Agreement between first and second genotyping was 83.7% (κ = .74, p < .001). For the 16.3% where the two rounds of genotyping proved discrepant, a third round was conducted that determined what genotype these discrepant cases would be assigned. Genotype distribution in the final sample (l/l: 28.1%; l/s: 50.2%; s/s: 21.7%) conformed to the Hardy–Weinberg Equilibrium (p = .98).

Data Analysis Plan

Data analysis focused on testing the moderating effect of DRD4 and 5-HTTLPR on the long-term associations between child-care experiences during the first 4.5 years and children's externalizing behavior from that age through spring of sixth grade and children's social skills from kindergarten through spring of sixth grade. An analytic strategy similar to that used by Belsky et al. (2007) and Pluess and Belsky (2010) was implemented. HLM (Bryk & Raudenbush, 2002; Singer & Willett, 2003) were fitted to estimate individual and group linear and quadratic growth curves. The models included family and child-care or school experiences measured during both the preschool (i.e., 0–54 months) and primary-school years (i.e., 54 months–sixth grade) as covariates, as well as child DRD4, 5-HTTLPR, and the two-way interactions involving each polymorphism and each of the three child-care variables (i.e., quality, quantity or hours, and center-care experience). Separate models were run to test each possible two-way interaction term between each gene and each child-care variable on both outcomes of interest, yielding a total of 12 separate models. Individual intercepts and linear slopes with respect to age were estimated as correlated random effects for each child. In the interest of space, reporting of results focuses exclusively upon the interactions between DRD4 and early child-care experiences, that is, those effects that extend previous findings (Belsky et al., 2007; Pluess & Belsky, 2010) are of primary interest here and proved statistically significant.

Several modeling decisions were made. First, age was centered at 54 months for externalizing

problems and at kindergarten for social skills, as these were the initial occasions at which the problem-behavior and social-skills measures that were repeatedly administered were obtained. Perhaps noteworthy, then, is the fact that these first measurements of the two constructs were provided by different respondents—child-care caregivers at 54 months in the case of behavior problems and kindergarten teachers in the case of social skills. The initial interaction effect coefficients to be presented indicate whether the G×E interactions of interest were related to (a) behavior problems at the age of 54 months or social skills at kindergarten and (b) change—or slope—of externalizing behavior from 54 months to sixth grade or of social skills from kindergarten to sixth grade. If any interaction term predicted the slope, the initial analysis was rerun repeatedly after changing the intercept age so that the changing nature over time of the interaction between the polymorphism and child-care factor in question could be illuminated.

Whenever GXE interactions predicting an intercept proved significant, we conducted a "regions-of-significance" analysis, following Kochanska, Kim, Barry, and Philibert (2011), to evaluate whether the significant interaction proved more consistent with a diathesis-stress or differential-susceptibility model of environmental action. The region of significance defines the specific values of a child-care feature (i.e., quality, quantity, type) at which the slope between a particular polymorphism and externalizing problems moves from significance to nonsignificance and/or vice versa (Aiken & West, 1991; Hayes & Matthes, 2009; Preacher, Curran, & Bauer, 2006). If and when evidence emerged from the regions-of-significance analysis that the data conformed to a differential-susceptibility model of environmental action, we double checked this conclusion by implementing a series of additional and more demanding analytic steps recently outlined by Roisman et al. (2012) for evaluating the nature of the interaction detected.

Missing data occurred in the included sample (n = 508) due to attrition and failure to complete all assessments, as follows with respect to the primary child-care predictors and child-development outcomes: quality of care (8.7%), hours of care (1.2%), center-care experience (2.6%), externalizing behavior (7.3%–30.3%, representing lowest and highest at any one time across repeated measurement occasions), and social skills (8.1%–15.4%). Missing data also occurred in primary-grade covariates, as follows: parenting quality (.6%–5.9%), maternal depression (1.0%–6.9%), income (3.0%–8.7%), partner presence (0.2%–5.5%), classroom quality (3.7%–20.9%), and after-school activities (1.6%–5.3%). Missing data of the included sample were imputed with multiple imputation (Schafer, 1997) using all available data (N = 1,364). Test statistics and regression coefficients were averaged across five imputed data sets. When analyses were run with only cases with complete data, results did not differ from those derived from the imputed data sets. For the simple slopes and regions-of-significance follow-up analyses, intercepts for externalizing behavior and social skills were estimated by means of HLM analyses. These intercepts were then averaged across the five imputed data sets. The level of significance for all analyses was set at $\alpha = .05$.

Results

Four sets of results are presented, the first preliminary, provides simple descriptive statistics on the measurements included in this report, while illuminating the most important bivariate relations among variables; the second primary, addressing the interactions of interest between DRD4 or 5-HTTLPR and child-care experiences in the prediction of children's externalizing behavior and social skills over time; the third, follow-up analyses illuminating the form of significant interactions; and a fourth set evaluating whether the detected G×E interactions remained significant when a previously chronicled interaction involving child care and difficult temperament was taken into account (Belsky & Pluess, 2012; Pluess & Belsky, 2009, 2010).

Preliminary Analysis: Descriptive Statistics and Unadjusted Associations

Table 1 presents the means and standard deviations of the variables of primary interest. With respect to the intercorrelation of these measurements, perhaps what is most important to note is that DRD4 and 5-HTTLPR were not significantly associated with child-care predictors or the child-development outcome variables except as follows: DRD4 with sixth-grade externalizing behavior ($r = -.10$, $p < .05$) and 5-HTTLPR with fifth-grade externalizing behavior ($r = -.09$, $p < .05$) and social skills ($r = .10$, $p < .05$). Given the lack of significant associations between genes and outcomes at all other assessment points, the data appear to fulfill

the criteria of independence of the moderator variable (i.e., DRD4, 5-HTTLPR) from the environment (i.e. child care) and outcome (i.e., externalizing problems, social skills), thereby generally meeting criteria for testing differential susceptibility (Belsky, Bakermans-Kranenburg, et al., 2007). Externalizing behavior and social skills were significantly and negatively associated at all assessments (range $r = -.13$ to $-.60$, $p < .01$).

Primary Analyses

For the primary analyses predicting, first, externalizing behavior and, second, social skills, we ran HLM across the multiple assessment points. Variables included child DRD4 and 5-HTTLPR, child-care quality intercept (estimated quality of care at 27 months, reflecting the midpoint between 6 and 54 months), the hours per week intercept (estimated from HLM analyses in which the intercept was set at 27 months), proportion of 3- to 4-month epochs in center-based child care for at least 10 hr per week, six preschool time-invariant covariates (child gender, infant temperament at 6 months, maternal education at month 1, mean income-to-needs ratio between 1 and 54 months, mean parenting quality between 6 and 54 months, mean maternal depressive symptoms between 6 and 54 months), and five concurrent time-varying covariates from 54 months through sixth grade (income-to-needs ratio, parenting quality, maternal depression, observed school classroom quality, and hours per week of after-school care (set to 0 for 54 months). The model was run once including only the covariates, child-care variables, and the two polymorphisms, all as main effects, and then again with two-way interactions involving DRD4 and 5-HTTLPR with each of the three child-care variables (i.e., six separate models for each outcome). Because 5-HTTLPR did not significantly predict the intercept or slope of either externalizing behavior or social skills, whether as a main effect or in interaction with any of the child-care variables, reporting below pertaining to genetic findings is restricted to those involving DRD4; Tables 2 and 3 present results for externalizing problems and social skills, respectively.

Externalizing Behavior

With regard to main effects, findings displayed in Table 2 indicate that externalizing problems at 54 months were significantly greater when parenting quality across the early years was lower, when children lived in a single-parent home (i.e., less partner presence), when children were exposed to higher proportions of center care, and when they spent more hours in child care irrespective of type of care. The amount of time spent in child care also significantly predicted the decline in externalizing behavior across the primary grade years. None of the time-variant primary-grade covariates significantly predicted the intercept or slope of externalizing behavior problems except after-school experience, which predicted both the intercept ($B = -0.06$, 95% CI [−0.12, −0.003], $p = .04$) and the slope ($B = 0.03$, 95% CI [0.01, 0.05], $p < .01$). Notably, neither DRD4 nor child-care quality significantly predicted problem behavior (intercept or slope) as main effects.

DRD4 and child-care quality did interact, however, to significantly predict externalizing behavior centered at 54 months ($B = -6.23$, 95% CI [−12.26, −0.21], $p = .04$) and to marginally predict the slope of externalizing behavior across the eight assessment points ($B = 0.96$, 95% CI [−0.11, 2.03], $p = .08$). The latter result was followed up below to determine whether the G×E in question remained significant at times of measurement after 54 months of age. The slope of externalizing problems was also significantly predicted by interactions involving DRD4 and child-care hours ($B = -0.02$, 95% CI [−0.04, −0.003], $p = .02$) and child-care type ($B = -0.97$, 95% CI [−1.88, −0.06], $p = .04$), but because follow-up analyses never revealed significant relations between these interactions and externalizing intercepts at any of the ages of measurement, in the interest of space, details of such analyses are not reported.

Social Skills

Results pertaining to main effects indicated that social skills at kindergarten were significantly greater when parenting quality across the early years was higher and when children did not live in a single-parent home (i.e., more partner presence). None of the time-variant primary-grades' covariates significantly predicted the intercept or slope of social skills except after-school experience, which predicted the slope ($B = -0.04$, 95% CI [−0.08, −0.005], $p = .02$). Notably, neither DRD4 nor any of the child-care variables predicted the social skills intercept at kindergarten as main effects.

DRD4 and child-care quality did interact, however, to predict social skills centered at kindergarten ($B = 10.67$, 95% CI [1.23, 20.11], $p = .03$)

Table 2
Summary of Hierarchical Linear Model Predicting Behavior Problems (N = *508*)

Predictor variables	Behavior problems intercept centered at 54 months *B*	Behavior problems slope *B*
Step 1		
Maternal education at 1 month	0.06	−0.04
Income-to-needs ratio (across 54 months)	0.18	−0.05
Maternal depression (across 54 months)	−0.03	< −0.01
Parenting quality (across 54 months)	−2.63**	0.03
Partner presence (across 54 months)	−0.05**	< 0.01
Child temperament at 6 months	−0.17	−0.18
Child gender (1 = *male*; 2 = *female*)	−0.02	0.02
Child-care quality (across 54 months)	0.02	0.37
Child-care hours (across 54 months)	0.08**	−0.01**
Center child care (across 54 months)	4.00**	−0.28
Child DRD4 (0 = *7R absent*, 1 = *7R present*)	0.59	−0.21
Child 5-HTTLPR (0 = l/l; 1 = l/s; 2 = s/s)	−0.15	−0.18
Step 2		
DRD4 × Child-Care Quality	−6.23*	0.96†
DRD4 × Child-Care Hours	0.07	−0.02*
DRD4 × Center Child Care	2.66	−0.97*

Note. The model included the following time-variant covariates from 54 months through sixth grade that are not displayed in the table: income-to-needs ratio, maternal depression, parenting quality, partner presence, classroom quality, after-school activities. The displayed coefficients of the variables at Step 1 represent the values before inclusion of interaction terms at Step 2.
†$p < .10$. *$p < .05$. **$p < .01$.

Table 3
Summary of Hierarchical Linear Model Predicting Social Skills (N = *508*)

Predictor variables	Social skills intercept centered at kindergarten *B*	Social skills slope *B*
Step 1		
Maternal education at 1 month	0.49†	−0.04
Income-to-needs ratio (across 54 months)	−0.15	0.08
Maternal depression (across 54 months)	0.03	< 0.01
Parenting quality (across 54 months)	3.60*	0.03
Partner presence (across 54 months)	0.06*	< 0.01
Child temperament at 6 months	0.81	0.15
Child gender (1 = *male*; 2 = *female*)	1.34	−0.40†
Child-care quality (across 54 months)	1.85	−0.12
Child-care hours (across 54 months)	−0.04	0.01
Center child care (across 54 months)	−3.25	0.73
Child DRD4 (0 = *7R absent*; 1 = *7R present*)	−0.46	0.18
Child 5-HTTLPR (0 = l/l; 1 = l/s; 2 = s/s)	−0.02	0.13
Step 2		
DRD4 × Child-Care Cuality	10.67*	−2.48*
DRD4 × Child-Care Hours	−0.01	< 0.02
DRD4 × Center Child Care	5.45	−0.82

Note. The model included the following time-variant covariates from kindergarten through sixth grade that are not displayed in the table: income-to-needs ratio, maternal depression, parenting quality, partner presence, classroom quality, after-school activities. The displayed coefficients of the variables at Step 1 represent the values before inclusion of interaction terms at Step 2.
†$p < .10$. *$p < .05$.

and the slope of social skills across the eight assessment points ($B = -2.48$, 95% CI [−4.52, −0.44], $p = .02$). The latter result was followed up below to determine whether the G×E in question remained significant at times of measurement after kindergarten.

Secondary and Tertiary Analyses: Form and Timing of G×E Interaction

Two sets of follow-up analyses were carried out to illuminate the significant DRD4 × Child-Care Quality interactions, one pertaining to the form of the interaction and the other to its potentially changing nature over time.

Interaction Form

To interpret the significant DRD4 × Child-Care Quality interaction in predicting the externalizing-behavior intercept at 54 months and the social-skills intercept at kindergarten, we plotted regression slopes of child-care quality on predicted externalizing problems at 54 months and social skills at kindergarten, separately for children with and without the DRD4 7-repeat allele. Figure 1 indicates that the relation between child-care quality and externalizing problems was negative and significant in the case of children carrying the DRD4 7-repeat allele ($\beta = -.27$, $p < .01$), but not in the case of children without the DRD4 7-repeat ($\beta = -.07$, $p = .21$). Figure 2 indicates that the relation between child-care quality and social skills was positive and significant in the case of children carrying the DRD4 7-repeat allele ($\beta = .35$, $p < .01$), but not in the case of children without the DRD4 7-repeat ($\beta = .10$, $p > .05$).

Visual inspection of Figures 1 and 2 reveals a crossover interaction consistent with differential-susceptibility in that children carrying the DRD4 7-repeat allele had the most externalizing problems when exposed to low-quality child care early in life yet the least such problems when quality was high, as well as the most social skills when quality was high and the least when quality was low. Analysis of regions of significance of the data on which Figure 1 is based, using a tool provided by Preacher et al. (2006), yielded only a lower bound of significance within the observed range of child-care quality, however; that is, the slope between DRD4 and externalizing problems proved significant when child-care quality was lower than 2.45 (i.e., shaded areas in Figure 1), representing 6.5% of the sample, with no significant differences emerging above this value. Analysis of the data on which Figure 2 is based, on the other hand, yielded both a lower and a higher bound of significance within the observed range of child-care quality; more specifically, the slope between DRD4 and social skills proved significant when child-care quality was lower than 2.53, representing 9.3% of the sample, and higher than

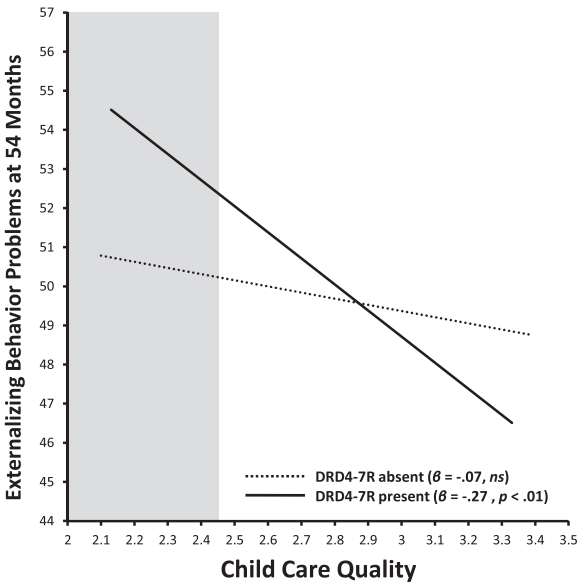

Figure 1. DRD4 × Child-Care Quality interaction predicting teacher-reported externalizing problems at 54 months. The shaded area represents the region of significance.

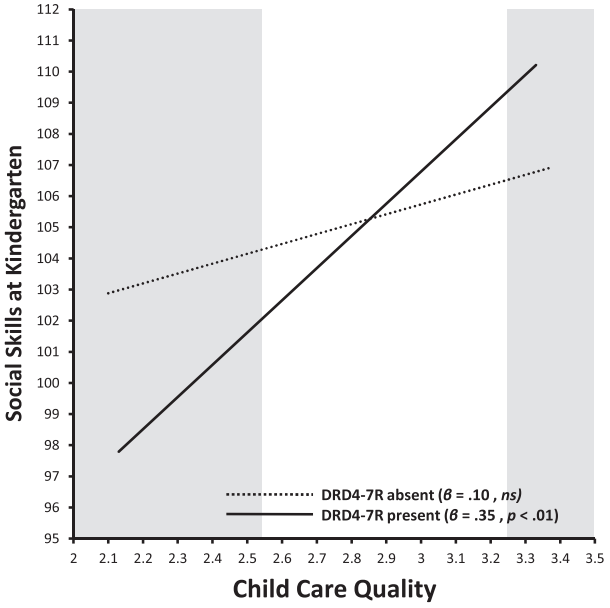

Figure 2. DRD4 × Child-Care Quality interaction predicting teacher-reported social skills at kindergarten. The shaded area represents the region of significance.

3.24, representing 2.2% of the sample (i.e., shaded areas in Figure 2). Consequently, the significant interaction between DRD4 and child-care quality in the prediction of externalizing problems at 54 months

was more consistent with a diathesis-stress model of environmental action, whereas the significant interaction between DRD4 and social skills at kindergarten was more consistent with a differential-susceptibility model.

Before confidently embracing a differential-susceptibility conclusion with respect to social skills at kindergarten, we proceeded to implement a series of additional analyses that Roisman et al. (2012) proposed for evaluating differential-susceptibility (using an Internet tool: http://www.yourpersonality.net/interaction/ros2.pl). According to these additional analyses, the crossover point of the simple slopes on child-care quality was at 2.85, well within their criterion range of ± 2 *SD* (*SD* = .23) from the mean (*M* = 2.82). The proportion of the interaction (PoI) provides a way to express the proportion of the total interaction that is represented below and above the crossover point, with PoIs close to .50 regarded by Roisman et al. as strong evidence of differential susceptibility. PoI in the ± 2 *SD* range with respect to social skills at kindergarten was .57 below and .43 above the crossover point, clearly close to the .50 criteria. The proportion affected (PA) index represents the proportion of cases that fall above the crossover point with larger percentages suggesting stronger evidence for differential-susceptibility relative to diathesis stress. PA with respect to social skills was .49. Hence, these additional analyses suggest that the interaction between child-care quality and DRD4 in the prediction of social skills does reflect a differential-susceptibility rather than a diathesis-stress pattern of environmental action, consistent with the visual interpretation of the regions-of-significance analysis.

G×E interaction over time. To illuminate the nature of the significant interaction between DRD4 and child-care quality in predicting the slope of behavior problems and of social skills, we ran, respectively, six or seven additional HLM models, each centered at a different assessment point (externalizing: kindergarten and first through sixth grade; social skills: first through sixth grade). After being significant at 54 months (see above), the interaction between DRD4 and child-care quality in the prediction of behavior problems proved marginally significant at kindergarten ($B = -5.35$, 95% CI [−10.83, 0.13], $p = .06$) and nonsignificant thereafter (*B*s = −4.00 to 0.68, $p > .10$ for all). Similarly, after being significant at kindergarten (see above), the interaction between DRD4 and child-care quality in the prediction of social skills proved marginally significant at first grade ($B = 7.45$, 95% CI [−0.56, 15.46], $p = .07$) and nonsignificant thereafter (*B*s = 4.89 to − 4.90, $p > .10$ for all). In both cases, then, the data suggest that the genetic moderation of effects of early child-care quality on children's social-emotional adjustment dissipated over time.

Including Child-Care Quality × Difficult Temperament Interaction

Recall that in earlier analyses of NICHD study data we discerned significant interactions between infant temperament and child-care quality in predicting behavior problems and social skills (Belsky & Pluess, 2012; Pluess & Belsky, 2009, 2010). Given the aforementioned evidence indicating that DRD4 is related to infant negative emotionality (Holmboe et al., 2011; Ivorra et al., 2010), we sought to determine whether the DRD4 × Child-Care Quality interactions reported herein were a function of the previously detected Temperament × Child-Care Quality interaction effects. Thus, we reran the two HLM with the significant interaction term between DRD4 and child-care quality predicting (a) externalizing behavior problems at 54 months and (b) social skills at kindergarten with an additional two-way interaction term involving difficult infant temperament and child-care quality added to the model (along with the main effect of temperament). Consistent with earlier findings (Belsky & Pluess, 2012; Pluess & Belsky, 2009, 2010), the interaction between temperament and child-care quality predicting the 54-month problem-behavior intercept proved significant ($B = -10.68$, 95% CI [−17.79, −3.57], $p < .01$), while the G×E interaction between DRD4 and child-care quality remained marginally significant ($B = -5.50$, 95% CI [−11.48, 0.47], $p = .07$). Similarly, the interaction between temperament and child-care quality predicting the kindergarten social-skills intercept proved marginally significant ($B = 10.82$, 95% CI [−1.04, 22.69], $p = .07$), whereas the G×E interaction between DRD4 and child-care quality remained significant ($B = 9.96$, 95% CI [0.51, 19.41], $p = .04$). These findings suggest that that the moderating effects of infant temperament and DRD4 vis-à-vis child-care quality and its effects on problem behavior and social skills are largely, even if not entirely, independent.

Discussion

The research reported herein had multiple goals, each of which is considered in turn before turning to study limitations.

G×E Interaction and Its Form

In this first study investigating whether effects of day care on children's social adjustment across the childhood years—reflected in their externalizing problems and social skills—might be genetically moderated, evidence emerged that this was indeed the case, but only with regard to DRD4, not 5-HTTLPR and quality of care. Recall that DRD4 and 5-HTTLPR were selected for inclusion in this research because it is these two polymorphisms for which the most G×E evidence has emerged seemingly consistent with differential susceptibility. It should be appreciated, however, that this could itself be an artifact of these two polymorphisms being among the most studied from a G×E perspective. It was somewhat surprising that no significant G×E interactions emerged in the case of 5-HTTLPR given the results of a recent meta-analysis evaluating—and documenting—such effects in the case of children under 18 years of age; indeed, it provided evidence of G×E consistent with differential susceptibility (van IJzendoorn et al., in press). It did not include, however, any research evaluating child-care effects.

With regard to the G×E effects detected, these involved DRD4 and quality of care predicting externalizing problems at 54 months and kindergarten and social skills at kindergarten and first grade. One of the things that makes such findings noteworthy is that different respondents provided evaluations of child behavior across the years of measurement covered in this inquiry: caregivers at 54 months, kindergarten teachers during the 1st year of school, first-grade teachers during the 2nd year, and so on through sixth grade.

The graphical plotting of the detected G×E interactions highlighting a crossover-interaction pattern appeared more consistent with differential-susceptibility rather than diathesis-stress thinking. The regions-of-significance analysis indicated otherwise, however, in the case of externalizing problems. Recall that it was only at low levels of quality of care that differences between children with and without the 7-repeat allele manifested themselves, with those carrying the allele appearing more adversely affected by low-quality care (i.e., manifesting more behavior problems), at least during the first 2 years that each outcome was measured, than those not carrying this allele. The fact that the regions-of-significance analysis also revealed that children carrying the 7-repeat allele who were exposed to higher quality care benefited more than other children from such supportive environments when the outcome to be explained was social skills proved consistent with the differential-susceptibility hypothesis. That is, there was evidence of both "for-better-*and*-for-worse" effects of child-care quality on children carrying the 7-repeat allele.

Of note, however, is that the percentage of children exposed to quality of care at a level that yielded a reliable developmental benefit—as revealed by the regions-of-significance analysis (see Figure 2)—was relatively small (2.2%), clearly raising questions about just how strong was the "for better" side of the differential-susceptibility equation in this instance. Nevertheless, when the crossover interaction indicative of differential-susceptibility was evaluated further by applying Roisman et al.'s (2012) evidentiary standards, the data continued to highlight the differential-susceptibility nature of the interaction under consideration.

Beyond Main Effects

On the basis of the findings just summarized, it would seem that prior analyses by the NICHD ECCRN of child-care effects and, in particular, quality-of-care effects on social adjustment from 54 months through sixth grade failed to detect any such effects because the original investigators only focused on main effects or effects moderated by child gender and family risk conditions (Belsky et al., 2007; NICHD ECCRN, 2003). Apparently—given the nonexperimental nature of the NICHD Study—low-quality child care does matter with respect to problem behavior and social skills around the time of the transition to school, but only for some children, not others, with the same perhaps being true of high-quality care in the case of social skills. If such findings can be replicated, they would call into question the widespread presumption that poor quality of care compromises the development of most children, at least with respect to socioemotional functioning, and that good quality care yields measurable benefits in this realm of functioning for most children. But even if this proves to be the case in future work, it would not provide grounds for reducing investment in quality of child care. Not only does the NICHD study and many others consistently find quality of care to (modestly) predict cognitive and language development (NICHD ECCRN, 2006), as well as academic achievement (Belsky et al., 2007; Vandell et al., 2010), but humanitarian considerations alone dictate the provision of good quality care conditions for all children.

The Moderating Effect of Difficult Temperament

Because previous analyses of NICHD study data indicated that quality effects on problem behavior or social skills were moderated by difficult infant temperament, with infants scoring high in negativity proving more susceptible—for-better-*and*-for-worse—to the apparent influence of child-care quality than other infants, the issue arose as to whether the G×E interactions discerned in this inquiry involving DRD4 could underlie—and account for—the parallel interactions involving temperament reported by Pluess and Belsky (2009, 2010). As it turned out, this did not prove to be the case. Recall that in the final set of analyses presented, both interactions involving quality of care proved significant or marginally so. This means that (a) infants with difficult temperaments or (b) children carrying the 7-repeat DRD4 allele are disproportionately, even exclusively, susceptible to effects of low-quality care on externalizing problem behavior and social skills around the transition to school.

To our knowledge, this is the first demonstration in a single inquiry of independent interactions involving temperament and genetics with the same (or even a different) environmental factor. There would seem to be reason to question, then, any conceptual privilege granted genes over temperament (or physiology; Boyce & Ellis, 2005) when it comes to considering organismic factors that might moderate environmental effects on human development, either in diathesis-stress or differential-susceptibility terms. It should be acknowledged, nevertheless, that results pertaining to the issue of overlap between interactions between child-care quality and temperament and DRD4 on which this conclusion is based might have been different had other measures of temperament, perhaps ones based on observations rather than maternal reports, been subject to analysis. Only future research will be in position to address this possibility.

Developmental Analysis

Besides focusing on the genetic moderation of child-care effects and distinguishing differential-susceptibility from diathesis-stress models of environmental action, one of the strengths of the current inquiry was that it was developmental in character. Because externalizing problems and social skills were repeatedly measured—by independent evaluators (i.e., different teachers each academic year)—we were in position not just to determine whether child-care effects were moderated by the child's genetic makeup at some point in time, but whether the G×E effect initially detected in problem behavior at 54 months and in social skills at kindergarten endured, dissipated, or even strengthened over time. This we could do by predicting in the initial model the slope of externalizing problem over time, that is, change in externalizing problems, and then shifting the intercept predicted in subsequent rounds of analysis from 54 months to kindergarten to first grade all the way through sixth grade.

As it turned out, the G×E interactions proved significant at the initial occasion at which the outcome was measured, but not thereafter. This seems likely to have been the result of experiences—in the family, at school, and in after-school programs perhaps—that intervened between when child-care experiences and later assessments of child functioning were obtained. Clearly, development is not over by the time children start school.

This observation highlights the need to avoid the implicit presumption that a G×E interaction (of any kind) detected at one point in time, as is the typical practice in most G×E inquiry, necessarily endures over time. At the same time, investigators should be alert to the possibility, not discerned in this inquiry, that a failure to detect such an interaction at one point in time does not automatically imply that it could not emerge subsequently. Only by considering functioning at multiple points in time and examining G×E effects on slope or change over time in a developmental outcome of interest will it be possible to address these matters.

Study Limitations

Perhaps the two biggest limitations of the current inquiry are that it was nonexperimental in nature and included only 508 of some 1,364 children initially enrolled in the NICHD study. The observational character of this investigation clearly limits any and all causal inferences that can be drawn from the findings presented. Nevertheless, language pertaining to child-care "effects" was employed throughout this report for heuristic purposes. A further, yet minor, limitation is that the study did not genotype SNP rs25531 and differentiate between L_A and L_G alleles, and thus we were not positioned to treat the L_G alleles as short alleles (Hu et al., 2006). One can also wonder whether, had the genotyping proven more reliable, more evidence of G×E interactions might have emerged, especially after the initial time of outcome measurements, or whether they might have proven more consistent with differential susceptibility rather than diathesis stress.

With regard to the latter, it also seems possible that greater inclusion of exceptionally high-quality child-care experiences might have resulted in findings more consistent with differential susceptibility.

The limited number of research participants included in this research was mostly due to the fact that not all families agreed to contribute DNA on their child and that 2 of the 10 participating research sites could not secure ethics' approval to request DNA from study participants' families. There was also a need, based on racial differences in gene frequencies, to keep the sample racially homogenous. Due to the consequential substantial reduction in sample size, it is difficult to be sure that the results presented herein would generalize to the original sample, to say nothing of a nationally—or internationally—representative one. Especially important in this regard is that the NICHD study children not included in this inquiry differed from those who were included in terms of their child-care experience and their social functioning.

In some sense, then, the results of this study should be regarded more as "proof of concept" rather than a basis for definitive conclusions. Results suggest that at least some child-care effects may vary across children as a result of their genetic makeup. They further provide evidence that G×E effects detected at one point in time may or may not endure over time. They additionally indicate that moderational effects involving temperament or any other child characteristic should not be presumed to reflect some underlying genetic factor, at least not without evidence that the moderating effect of a temperamental factor is accounted for by the moderating effect of a genetic one.

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