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Child μ-Opioid Receptor Gene Variant Influences Parent–Child Relations

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Variation in the μ-opioid receptor gene has been associated with early social behavior in mice and rhesus macaques. The current study tested whether the functional *OPRM1* A118G predicted various indices of social relations in children. The sample included 226 subjects of self-reported European ancestry (44% female; mean age 13.6, SD = 2.2) who were part of a larger representative study of children aged 9–17 years in rural North Carolina. Multiple aspects of recent (past 3 months) parent–child relationship were assessed using the Child and Adolescent Psychiatric Assessment. Parent problems were coded based upon a lifetime history of mental health problems, substance abuse, or criminality. Child genotype interacted with parent behavior such that there were no genotype differences for those with low levels of parent problems; however, when a history of parent problems was reported, the G allele carriers had more enjoyment of parent–child interactions (mean ratio (MR) = 3.5, 95% CI = 1.6, 8.0) and fewer arguments (MR = 3.1, 95% CI = 1.1, 8.9). These findings suggest a role for the *OPRM1* gene in the genetic architecture of social relations in humans. In summary, a variant in the μ-opioid receptor gene (118G) was associated with improved parent–child relations, but only in the context of a significant disruption in parental functioning.

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INTRODUCTION

Binding of the endogenous opioids to the μ-opioid receptor (Bodnar, 2008; Kieffer and Evans, 2009; Kreek and LaForge, 2007) activates reward systems by modulating circuitry in the ventral tegmental area (Spanagel *et al*, 1992) and nucleus accumbens (Simmons and Self, 2009). As such, it is a critical player in reinforcement of both natural and artificial rewards. Among the behavioral systems influenced by the μ-opioid receptor is the infant social attachment system (Nelson and Panksepp, 1998). During periods of interaction with a caregiver, opioids are released, thereby contributing to reinforcement of the attachment bond. A relative reduction in opioid release, which occurs during periods of separation, increases an infant's motivation to seek and maintain proximity to its caregiver (Herman and Panksepp, 1978; Kalin *et al*, 1995; Knowles *et al*, 1989). The importance of endogenous opioids for attachment-related behaviors have been validated pharmacologically, as both attachment and separation responses are attenuated with nonsedating doses of the μ-opioid receptor agonist, morphine (Kalin *et al*, 1988; Panksepp *et al*, 1978).

Genetic variation that affects μ-opioid receptor function has been demonstrated to influence social behavior in various animal models (Barr *et al*, 2008; Moles *et al*, 2004). Mice lacking the *OPRM1* receptor gene show prominent deficits in maternal separation-induced ultrasonic vocalizations, preference for maternal cues, and ultrasonic call potentiation after brief maternal exposure (Moles *et al*, 2004). In rhesus macaques, a nonsynonymous SNP in the *OPRM1* gene (rh*OPRM1* C77G) that increases reward sensitivity (Barr *et al*, 2007) predicts increased vocalization during periods of maternal separation and social preference for the caregiver upon reunion (Barr *et al*, 2008). These data suggest that spontaneous genetic variation at the *OPRM1* gene might influence the development of social attachment and other related phenotypes in humans.

In humans, there is a nonsynonymous SNP (*OPRM1* A118G) that results in an amino-acid substitution in the N-terminal arm of the receptor. This genetic variant has been shown to predict increased response to 'reward' in a variety of paradigms. Recent studies also show increased sensitivity to social rejection in G allele carriers (Way *et al*, 2009).

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Originally considered a gain-of-function allele (Bond *et al*, 1998), recent studies examining either *in vitro* properties or genetic association with substance disorders have produced mixed results (Arias *et al*, 2006; Kroslak *et al*, 2007). Given that functional variation appears to influence the development of social attachment in nonhuman species, we wanted to test whether there was an effect of *OPRM1* A118G on the quality of human parent–child relations. As studies in other species (Barr *et al*, 2008; Moles *et al*, 2004) suggest that genetic variation might produce effects as a function of repeat exposures to separation from a caregiver, we wanted to examine whether there were interactions of genotype with parental inconsistency or unavailability. In this study we examined whether *OPRM1* A118G genotype predicted parent–child relations, and whether there were interactive effects of genotype with significant disruption in parental functioning.

MATERIALS AND METHODS

Sample and Procedures

The Caring for Children in the Community study (CCC) is a representative study of psychiatric illness and service use in African-American and White youth in four rural counties in the southeast with high rates of poverty. The two-stage sampling design and methods are described in detail elsewhere (Angold *et al*, 2002). Briefly, 4500 youths were randomly selected from all 17 117 9–17 year olds in the public school's database. Of these, 3613 were contacted and agreed to complete screens (the externalizing scale of the CBCL). Of these families, 1302 were selected to participate in the interviews, and 920 (70.7%) interviews were completed.

Children were selected with different probabilities from each decile of CBCL scores. Participants were assigned a weight inversely proportional to their probability of selection, so that the results from our analyses reported in this study are representative of the original populations from which the samples were drawn. Parent and child signed informed consent/assent forms approved by the institutional review board of the Duke University Medical Center.

This study sample was limited to subjects of self-reported European ancestry ($N=337$) because the polymorphism of interest appears to be rare ($<1\%$) in African-derived populations (HapMap). Of the 337 subjects, 71% or 238 consented to provide blood samples using standard blood spot collection procedures. Spots from 12 subjects were too small for extraction. DNA was isolated from 226 dried blood samples using standard extraction procedures.

Measures

Primary outcomes. Both subjects and parents were interviewed with the Child and Adolescent Psychiatric Assessment (CAPA) (Angold and Costello, 2000). All outcomes were assessed over the preceding 3 months. This study did not include a formal assessment of attachment as currently conceptualized. The CAPA does query parents and child about three aspects of the relationship: (1) enjoyment of parent–child activities (scale from 0 to 2); (2) parent–child arguments (count of arguments); and (3) separation anxiety symptoms (count of eight possible symptoms). Assessment of enjoyment of parent–child activities focused on the extent of the time the interviewees enjoyed activities with their parent–child (ie, >75, 25–75, or $<25\%$). When both parent and child report was available, values were averaged for parent–child relation and their arguments. Assessment of separation anxiety was particularly important for discriminating whether the parent–child bond was reinforced by positive affect, anxiety, or both. A separation anxiety symptom was counted as present if it was reported by the primary caregiver, child, or both, as is standard clinical practice. Construct validity of the CAPA, as judged by 10 different criteria including relation to diagnostic rates found using other interviews and relation of CAPA-identified disorders to mental health service use, was good to excellent (Angold and Costello, 1995, 2000).

Specificity analyses. To test whether the genetic effects were specific to parent–child relations, two additional outcomes that were related were included: symptoms of depression and conduct problems. It was hypothesized that *OPRM1* would not be associated with either of these outcomes. As with separation anxiety symptoms, depression and conduct symptoms were assessed with the CAPA and counted as present if reported by the primary caregiver, child, or both.

Parent problems. To test whether effects of the *OPRM1* G allele on social relations varied by parent behavior, a measure of parental impairment was derived. In animal studies, parental availability is easily manipulated. As this is not possible in human samples, parent problems were chosen that would predispose the parent to be inconsistent, impaired, or unavailable for parent–child relations (see, eg, Jaffee *et al*, 2006; Lieb *et al*, 2000; Marcenko *et al*, 2000). Parents were coded as likely to have been impaired if they reported *ever* having: (1) significant mental health problems requiring treatment (39.1%, $N=93$), (2) substance abuse problems requiring treatment (9.5%, $N=28$), or (3) a criminal conviction (42.9%, $N=90$). These items were assessed as part of the parental functioning portion of the CAPA interview with the parent (Angold and Costello, 2000). For the mental health and substance-related indicators, parents were asked about specific types of treatment that may have been required (eg, medication, hospitalization). The parental criminality questions include follow-up question about disposition. The κ statistics for 1-year agreement ranged from 0.39 to 0.46 for the three indicators. In all, 59.1% of children ($N=138$) had a parent reporting at least one of these problems, 25.6% ($N=59$) reported two or more of these problems, and 5.7% ($N=14$) reported all three problems.

Genotyping. The 5′ nuclease genotyping assay was used for genotyping. Locus-specific primers and fluorogenic allele-specific probes were obtained from ABI (Assays-on-Demand, identification no. C_8950074). Approximately 10 ng of genomic DNA was amplified by real time-polymerase chain reaction (RT-PCR), using allele-specific probes. The reaction mixture consisted of 5 μl of master mix, 0.25 μl of 20 × assay mix, and 10 ng of genomic DNA diluted in distilled water. Amplification was performed

(Gene Amp PCR system 97000; Applied Biosystems) using 384-well plates and the following amplification profile: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 92 and 60 °C for 1 min. After amplification, end point fluorescence intensity was measured directly in the reaction plates (7900 sequence detector; Applied Biosystems). Four genotyping clusters were identified: A homozygotes, AG heterozygotes, G homozygotes, and no-DNA template controls. Genotype completion and accuracy (based on 10% replicate samples) were both 100%. Results were reported blind to outcome data.

Analytic Strategy

To insure independent analyses, correlations were tested among the three parent–child relations variables. Substantial intercorrelations would suggest nonindependence. The main effect of genotype on each outcome was tested using weighted Poisson regression models. The generalized estimating equations (GEE) option was employed to adjust standard errors of the parameter estimates for the stratified design effects. All models were run in SAS using PROC GENMOD with the GEE option (SAS Institute, 2004). Each model tested genotype as a predictor, adjusting for the covariates of age and sex. For all analyses, the genotype had two levels: A/A and G allele carriers (collapsing heterozygotes and homozygotes). To test for an interaction between genotype and parent behavior, the parent problems variable was entered into the main effect model along with an interaction term between genotype and parent problems. If significant, the LSMEANS statement with the DIFF option was used to compute least-square means estimates for the particular interaction effects and to test differences between such effects.

RESULTS

Of the 226 subjects, 163 had the A/A genotype (weighted percent = 66.3), 58 were heterozygous for the G allele (28.7%), and 5 were G homozygotes (5.1%). All results are based upon models in which G/Gs were grouped with heterozygotes (see, eg, Arias *et al*, 2006). The pattern of results did not differ when analyzed under an additive (G homozygotes in a separate group) or when G homozygotes were excluded. The frequency of the G allele was 15.0%, comparable with the minor allele frequency of 16.7% for the European panel of HapMap (Gibbs *et al*, 2003). Genotype frequencies did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 0.004$, $p =$ NS). G allele rates were higher in males than females (10 *vs* 19%, $p = 0.03$), but genotype was not associated with age (G allele carrier mean = 13.1 (SD = 2.5) *vs* A/A mean = 13.7 (SD = 2.1), $p = 0.23$).

Current parent–child relations were assessed through parent and self-report of the following variables as they were characterized in the previous 3 months: separation anxiety symptoms, enjoyment of parent–child activities, and parent–child arguments (Table 1). The intercorrelations between outcomes were very modest (r range = 0.04–0.20).

OPRM1 genotype influenced all three measures of parent–child relations. After accounting for the effects of sex and age, the *OPRM1* 118G allele predicted increased enjoyment of parent–child activities ($\chi^2(1) = 6.93$, $p < 0.01$, means ratio (MR) = 2.44 95% CI = 1.17, 5.21), lower levels of separation anxiety symptoms ($\chi^2(1) = 3.93$, $p < 0.05$, MR = 0.55 95% CI = 0.31, 0.98), and fewer parent–child arguments ($\chi^2(1) = 86.34$, $p < 0.01$, MR = 0.59 95% CI = 0.54, 0.67). *OPRM1* genotype was not related to other emotional and behavior problems, such as symptoms of depression ($\chi^2(1) = 1.29$, $p = 0.26$, MR = 1.14, 95% CI = 0.82, 1.60) or conduct disorder ($\chi^2(1) = 0.75$, $p = 0.39$, MR = 1.16, 95% CI = 0.67, 1.99).

There were also interactive effects between parent problems and genotype. There was no significant difference in parental problems by child genotype (A/A: 59.0% ($N = 99$) *vs* G allele carriers: 60.5% ($N = 39$), $\chi^2(1) = 0.01$, $p = 0.92$), indicating that genotype was not a proxy for parent problem status. Among subjects whose parent(s) self-reported a history of significant problems, those who were carriers of the G allele displayed a pattern of parent–child relations that differed from that observed in A homozygotes. G allele carriers reported more enjoyment of interactions with parents. However, the effect of genotype was limited to subjects reporting parent problems (Figure 1, genotype by parent problems interaction: $\chi^2(1) = 6.6$, $p = 0.01$). A similar interaction was observed for parent–child arguments, with the G allele being associated with fewer parent–child arguments, but only as a function of

Table 1 Definitions and Descriptive Information About Outcomes ($N = 226$)

Outcomes	A/A ($N = 163$)	G allele carriers ($N = 63$)	Overall ($N = 226$)
Primary: parent–child relationship	Mean (SD)	Mean (SD)	Mean (SD)
Enjoyment of activities with parent: activities between child and his/her parents are rated according to the degree of pleasure, displeasure, or disinterest associated with them.	0.30 (1.06)	0.67 (1.17)	0.55 (1.16)
Parent–child arguments: number of parent–child arguments	9.07 (26.57)	5.4 (17.9)	7.83 (24.49)
Separation anxiety symptoms: DSM-IV symptoms of separation anxiety disorder	0.31 (0.83)	0.19 (0.77)	0.27 (0.81)
Secondary: prosocial behavior			
Depressive symptoms: non-overlapping DSM-IV symptoms of major depression or dysthymia	0.65 (1.18)	0.67 (1.13)	0.66 (1.16)
Conduct symptoms: DSM-IV symptoms of conduct disorder	1.43 (1.57	1.34 (1.53)	1.40 (1.56)

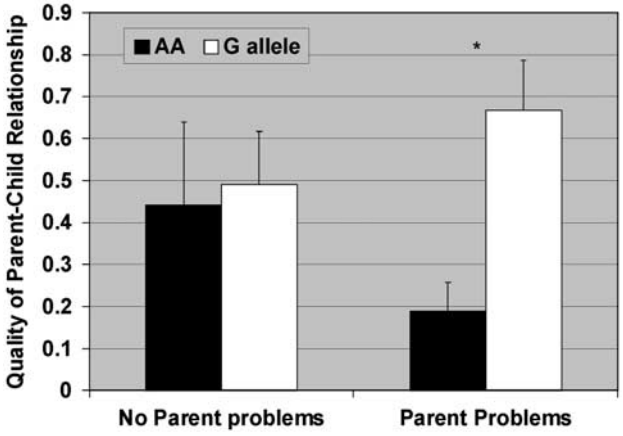

Figure 1 Results of the Poisson multiple regression analyses estimating the association between parent problems and enjoyment of parent–child interactions as a function of the *OPRM1* A118G genotype. The overall main effect for genotype was significant ($\chi^2(1) = 4.3$, $p = 0.04$, MR = 2.0, 95% CI = 1.0, 3.8), but not for parent problems ($\chi^2(1) = 0.7$, $p = 0.40$, MR = 1.31, 95% CI = 0.7, 2.5). The interaction term (genotype by parent problems) interaction term was also significant ($\chi^2(1) = 6.6$, $p = 0.01$). For those with no parent problems, genotype status is not significant ($\chi^2(1) = 0.04$, $p = 0.84$, MR = 1.1, 95% CI = 0.4, 3.1), whereas for those with parent problems, genotype predicts enjoyment of parent–child interactions ($\chi^2(1) = 9.5$, $p = 0.002$, MR = 3.5, 95% CI = 1.6, 8.0).

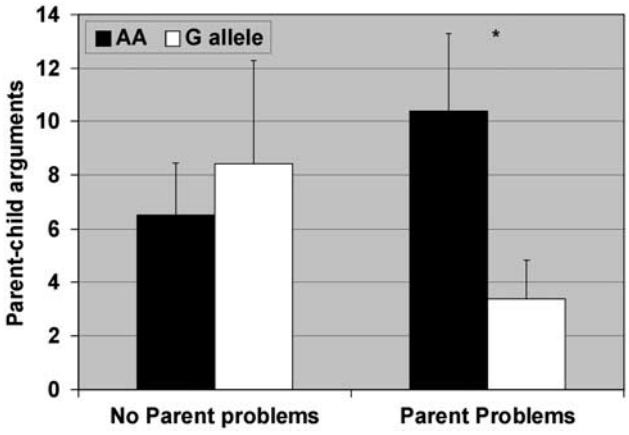

Figure 2 Results of the Poisson multiple regression analyses estimating the association between parent problems and parent–child arguments as a function of the *OPRM1* A118G genotype. Neither of the main effects were significant (genotype: $\chi^2(1) = 1.1$, $p = 0.30$, MR = 1.5, 95% CI = 0.7, 3.5; parent problems: $\chi^2(1) = 0.4$, $p = 0.54$, MR = 1.2, 95% CI = 0.6, 2.5). The interaction term (genotype by parent problems) interaction term was also significant ($\chi^2(1) = 4.7$, $p = 0.03$). For those with no parent problems, genotype status is not significant ($\chi^2(1) = 0.21$, $p = 0.65$, MR = 0.8, 95% CI = 0.2, 2.4), whereas for those with parent problems, genotype predicts enjoyment of parent–child interactions ($\chi^2(1) = 4.5$, $p = 0.03$, MR = 3.1, 95% CI = 1.1, 8.9).

parent problems (Figure 2, genotype by parent problems interaction: $\chi^2(1) = 4.7$, $p = 0.03$). The G allele did not interact with parent problems to predict the separation anxiety symptoms (Figure 3, genotype by parent problems interaction: $\chi^2(1) = 2.0$, $p = 0.16$). Follow-up analyses tested the influence of excluding a single indicator (mental health problems, substance problems, or criminality) from the parent problem variable. The pattern of findings did not vary with the omission of any of the specific parent problems.

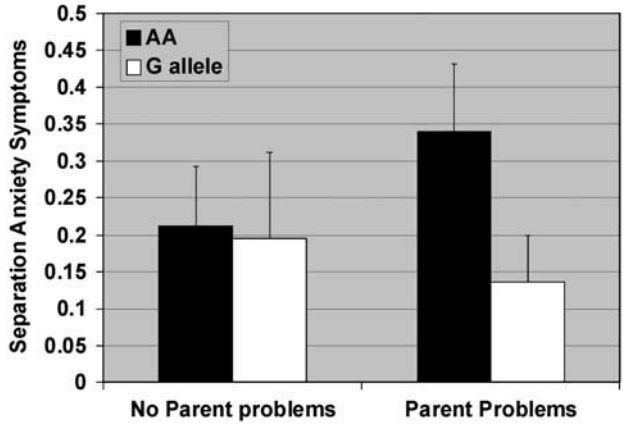

Figure 3 Results of the Poisson multiple regression analyses estimating the association between parent problems and separation anxiety symptoms as a function of the *OPRM1* A118G genotype. Neither of the main effects were significant (genotype: $\chi^2(1) = 1.3$, $p = 0.25$, MR = 1.6, 95% CI = 0.7, 4.0; parent problems: $\chi^2(1) = 0.0$, $p = 0.90$, MR = 0.9, 95% CI = 0.4, 2.3). The interaction term (genotype by parent problems) interaction term was not statistically significant ($\chi^2(1) = 2.0$, $p = 0.16$). For those with no parent problems, genotype status is not significant ($\chi^2(1) = 0.0$, $p = 0.91$, MR = 1.1, 95% CI = 0.3, 4.3), and for those with parent problems, genotype showed a trend toward predicting enjoyment of parent–child interactions ($\chi^2(1) = 2.9$, $p = 0.09$, MR = 2.5, 95% CI = 0.9, 7.4).

DISCUSSION

Understanding the genetic architecture of social relations is critical, given the role of interpersonal functioning in normal development and in the etiologies of almost every major psychiatric disorder (American Psychiatric Association, 1994). In this study, a common *OPRM1* variant influenced the quality of parent–child relationships, especially in the context of having a parent with a history of mental health problems, substance problems, or criminality. Each of these problems represents a potentially significant disruption in the parent's functioning and may be associated with a range of parenting problems (see, eg, Jaffee *et al*, 2006; Lieb *et al*, 2000; Marcenko *et al*, 2000). In these cases, *OPRM1* G allele carriers were advantaged across two measures of parent–child relations when compared with A/A subjects, but not in the case of separation anxiety symptoms.

The finding that the *OPRM1* 118G allele was protective in humans converges with results from a knockout study in mice (Moles *et al*, 2004) and our previous work on the rh*OPRM1* C77G SNP in macaques (Barr *et al*, 2007). This convergence is not trivial given the unavoidable methodological differences among the studies. The mouse study presented an extreme test of the importance of the μ-opioid receptor (ie, genetic deletion), whereas the macaque study focused on a functional SNP. In both studies, it was possible to control the subjects' environments to test attachment behavior under extreme conditions. The current study tested the role of a SNP within a heterogeneous group

of children and adolescents and relied upon natural variation in parent behavior rather than experimental control. Indeed, this study did not formally assess attachment as defined in developmental psychological literature, but parent–child relations. This study cannot be considered a 'replication' of the animal studies. Despite all of these differences, however, each subsequent study has observed the fundamental pattern of variations in caregiver–child interactions as a function of variation in the *OPRM1* gene.

Accumulating evidence suggests that oxytocin-, vasopressin-, and opioid-modulated mesocorticolimbic dopaminergic pathway activity accounts for the rewarding aspects of social interactions (Insel, 1997; Nelson and Panksepp, 1998). The endogenous opioids modulate reward pathways via activation of μ-opioid receptors in the ventral tegmental area (Spanagel *et al*, 1992) as well as the nucleus accumbens (Simmons and Self, 2009). This study supports the hypothesis that at least part of the rewarding/reinforcing aspects of social interactions in humans may be mediated by endogenous opioids. Furthermore, by demonstrating an interaction with parental availability or consistency, these findings reinforce observed similarities between patterns of parent–child interactions and substance withdrawal, wherein lack of positive reinforcement, either through removal of social interaction or the substance, is associated with behavior oriented toward the removed stimulus. This similarity is not unexpected if the positive effects of parent–child interaction are mediated by endogenous opioid binding to the μ-opioid receptor.

There are numerous reports suggesting a functional role for the *OPRM1* A118G SNP. However, the exact nature of that role remains unclear and is potentially complex. Originally proposed to be a gain-of-function mutation (Bond *et al*, 1998), subsequent studies suggest that the *in vitro* effects of the 118G polymorphism may depend on the cell line and/or outcome measures (Befort *et al*, 2001; Beyer *et al*, 2004; Kroslak *et al*, 2007). Moreover, studies examining *OPRM1* A118G genotype as a susceptibility factor for broad substance-related phenotypes are inconsistent (Arias *et al*, 2006). However, despite the inconsistencies listed above, a variety of studies demonstrate that the G allele behaves *in vivo* as a gain-of-function allele for opioid-modulated intermediate phenotypes, such as HPA-axis activity (Bart *et al*, 2006; Wand *et al*, 2002) pain threshold (Fillingim *et al*, 2005), and alcohol response (Ray and Hutchison, 2007; Ray, 2005). In fact, robust effects of *OPRM1* genotype have been found in functional studies focused on narrowly defined quantitative phenotypes that were most closely related to the proposed function of the genetic variant (see, eg, Wand *et al*, 2002; Bart *et al*, 2006; Fillingim *et al*, 2005; Ray and Hutchison, 2007). This study employed a similar approach. To reduce the signal-to-noise ratio, we also included multiple, independent indices of parent–child interaction phenotypes, tested the specificity of the effect by including secondary outcomes, and stratified based upon a environmental exposure known to affect parenting behavior.

There are a number of scenarios that may lead to false positives in genetic association studies (Sullivan *et al*, 2001). To guard against type I error, we (1) tested a hypothesis that has been previously supported in two species, (2) limited our focus to a single SNP with the strongest evidence of function, and (3) tested the robustness of the effect by looking at three relatively independent outcomes. Despite these safeguards, all genetic associations must be considered 'tentative information' until replicated in multiple independent samples (Ioannidis, 2006).

Another potential concern is stratification resulting from population admixture. To minimize this risk, only subjects of self-reported European ancestry were included and the larger epidemiological sample was collected from a rural area with no urban centers. Risk of experimental bias was minimized by blinded genotyping and regenotyping of 10% of all samples. It is important to keep in mind that parental behavior and parent–child interactions were assessed in the same interview. It is not possible, therefore, to clarify temporal sequence. At the same time, all parent–child outcomes were anchored to the 3 months immediately preceding the interview, whereas the parenting behavior variables (ie, mental illness, substance problems, and criminality) were based on whether they had ever occurred. The 1-year κ values for parent problems were relatively low and it is not possible to clarify the timing of the parent problem with respect to the child's birth. Finally, it is important to consider the possibility that *OPRM1* A118G may not be the functional variant driving the observed phenotypic differences, but rather a proxy marker for a functional haplotype.

The previous study in macaques speculated that genetic variation that increased reward sensitivity might have conferred a selective advantage at some point in evolutionary history of both rhesus and humans, by increasing attachment in response to caregiver unavailability. This study demonstrates an effect of *OPRM1* variation on development of social relations in humans and suggests that the same allele that has been proposed to increase risk for developing substance dependence in adolescence and young adulthood may be protective against poor parent–child relations in childhood. As such, this allele may provide a genetic basis for 'resilience' to impaired attachment in the face of a significant disruption in the parent's functioning.

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DISCLOSURE

The authors declare no conflict of interest.

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