Preadoption adversity, MAOA, and behavioral adjustment in internationally adopted Chinese girls
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Objectives We studied postinstitutionalized adopted Chinese girls to determine whether those with different variants of the MAOA gene promoter region (MAOA-VNTR) differed in their internalizing and externalizing behavior problems and whether the MAOA genotype moderated the relation between preadoption adversity and current behavior problems.

Methods MAOA genotyping was obtained for 94 girls (mean age: 9.2 years) and the number of 4-repeat (4R) alleles was determined (zero, one, or two). The adoptive mothers rated several indicators of preadoption adversity shortly after adoption (mean age at adoption 15.8 months) and completed the Child Behavior Checklist when the children were 8.1 years on average.

Results No main effects were found for preadoption adversity. However, the MAOA genotype had a nominally significant effect (P < 0.05) on internalizing problems. Regression analyses controlling for age, household income, authoritative parenting, and family problems showed that among children with no physical signs of preadoption adversity, those carrying a greater number of 4R alleles scored significantly lower (P < 0.01) on internalizing problems than those with fewer 4R alleles. Differences in internalizing scores related to the MAOA genotype were not observed for children who showed one or more physical signs of adversity at the time of adoption. A similar pattern was found for externalizing problems, although the results did not reach conventional levels of significance.

Conclusion Our results suggest that higher MAOA activity may be protective with respect to internalizing problems in internationally adopted Chinese girls, but that this protective effect decreases at higher levels of preadoption adversity. A similar pattern may exist for externalizing problems. Psychiatr Genet 24:211–217 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords: adopted children, behavior problems, early adversity, MAOA

Introduction Internalizing and externalizing problems in childhood are risk factors for adulthood mental disorders such as anxiety and depression (e.g. Roza et al., 2003; Carballo et al., 2011). For this reason, many researchers have explored both environmental and biological factors related to these types of problems in childhood and adolescence. For such contributors, starting with the Caspi et al. (2002) seminal study, a number of investigations have reported that early maltreatment interacts with the MAOA genotype to predict the risk of childhood behavioral problems.

The MAOA gene encodes an enzyme (MAOA) that metabolizes (and thus deactivates) several neurotransmitters (Caspi et al., 2002). This X-linked gene has a variable number tandem repeat (VNTR) polymorphism in the promoter region. Although most researchers consider the 4-repeat (4R) variant to be associated with a higher level of gene expression (i.e. higher ‘activity’) than the 3-repeat (3R) variant, there is some disagreement on the activity levels of the rarer VNTR alleles (2R, 3.5R, 5R).

In terms of their relationship with childhood behavior problems, one meta-analysis (Kim-Cohen et al., 2006) found that boys who carry a ‘high-activity’ variant (defined as 3.5R or 4R) have fewer externalizing problems than boys carrying a ‘low-activity’ variant (defined as 2R, 3R, or 5R). However, fewer studies have been carried out with girls and the findings have been more mixed. For example, Enoch et al. (2010) reported that among girls raised in stressful environments, those who carried a greater number of ‘high-activity’ alleles (defined as 4R) showed less hyperactivity than those carrying fewer ‘high-activity’ alleles. Similarly, Prom-Wormley et al. (2009) reported that girls carrying a greater number of ‘high-activity’ alleles (defined as 3.5R or 4R) showed lower levels of conduct disorder. However, other studies have found contrasting results. For example, one group of researchers has reported that preadolescent or adolescent girls with a greater number of ‘high-activity’ alleles (defined as 3.5R, 4R, or 5R) are at a higher risk for externalizing problems (e.g. delinquency) when exposed to early maltreatment (e.g. Oreland et al., 2007; Sjöberg et al., 2007; Aslund et al., 2011).
With respect to internalizing problems, very little research has been carried out with either males or females, and the results have also been mixed. Because MAOA inhibitors are commonly used to treat anxiety disorders (Tyrer and Shawcross, 1988), a link between naturally occurring MAOA activity and internalizing problems might reasonably be proposed. Consistent with this hypothesis, Yu et al. (2005) reported that a greater number of 4R alleles was associated with major depression in both male and female Chinese patients. However, Huang et al. (2009) found no significant association between depression and MAOA. In a study of low-SES American adolescents, Cicchetti et al. (2007) reported that ‘high-activity’ MAOA (3.5R, 4R or 5R) was associated with internalizing symptoms for nonmaltreated or moderately maltreated youth, but the opposite pattern was found for more severely maltreated youth. However, heterozygous females were excluded from their analyses and no sex differences were reported. Because depression rates are particularly high for females (as opposed to males; Keenan and Hipwell, 2005) and internalizing problems are a risk factor for depression, further study of the potential effects of MAOA on internalizing problems in girls would be particularly important.

One limitation in most existing studies of genes, environment, and behavioral problems in children lies in the fact that children typically remain in a similar environment throughout childhood and beyond. As a result, it is difficult to discern whether it is the experience of adversity specifically during their first few years or the cumulative experience of adversity across their entire childhood that may interact with MAOA in influencing later outcomes. To address this concern, some researchers have capitalized on a ‘natural experiment’ by studying children who were initially raised in institutions, but were later adopted internationally into high SES homes. Childcare institutions (e.g., orphanages) are undoubtedly a stressful environment for young children. However, for internationally adopted children, their adverse early experience is replaced by a favorable environment upon adoption, thus isolating psychosocial adversity within early childhood (Van der Vegt et al., 2009). In terms of links between MAOA and behavioral problems in post-institutionalized children, Van der Vegt et al. (2009) found that ‘high-activity’ MAOA (i.e. the 4R variant) was associated with greater externalizing problems in internationally adopted boys than was ‘low-activity’ MAOA (i.e. the 3R variant). This finding is in contrast to those generally reported for nonadopted boys experiencing prolonged stress or maltreatment. However, Van der Vegt et al. (2009) did not investigate adopted girls or internalizing problems.

Our study begins to address the need for further research that focuses on the interaction between MAOA and early (rather than continuous and persistent) childhood adversity, that focuses on girls and that measures internalizing as well as externalizing behavior problems. For this, we studied a sample of postinstitutionalized girls adopted from China. We asked (a) whether MAOA-VNTR variation might be associated with differences in adopted Chinese girls’ internalizing and externalizing behavior problems and (b) whether MAOA-VNTR variation moderated the relation between preadoption adversity and current behavior problems.

Methods
Participants
The children in this analysis (N = 94) were Han Chinese girls who were adopted into American families after spending a period of time in China’s Children’s Welfare Institutes. All were from the southern region of the PRC. The girls were from an ongoing longitudinal study on adopted Chinese children’s development and were adopted at 15.8 months on average (SD = 14.5). Because most Chinese boys available for international adoption have special needs, we included only girls in our sample.

Preadoption adversity
In 2005, when we launched the longitudinal study, the adoptive mothers provided demographic data and information about the children’s developmental and behavioral conditions around the time of adoption. Because direct access to institutions is rarely possible, parent report is commonly used to estimate the degree of children’s preadoption experience of adversity (e.g. Verhulst et al., 1992). However, in our study, we did not ask parents to ‘diagnose’ their children or provide direct estimates of their early adversity experiences. Instead, we conceptualized preadoption adversity as involving suboptimal experiences that would manifest in newly adopted children’s physical condition, developmental status, and social-emotional behaviors. Specifically, we collected survey data from the adoptive mothers in the following areas.

Signs and symptoms
Parents were presented with a list of 11 easily observable physical signs and symptoms (e.g., scars, sores, lice, untreated medical condition) generated from in-depth preliminary interviews with adoptive families and from an earlier study on 750 children adopted from China (Tan and Marfo, 2006). Parents were asked to check all signs/symptoms that they observed when the child was first adopted. Overall, 22.6% of the children had rashes, 11.9% had scars, and 9.5% had scratch marks, and a few children had other signs such as scabies. The total number of signs/symptoms was used to reflect one aspect of their preadoption adverse experiences. In data analysis, we truncated the total to range from 0 (no signs/symptoms observed) to 2 (two or more observed signs/symptoms).

Among the adoptees, 53.2% did not have any of the 11 signs/symptoms, 25.5% had one, and 21.3% had two or more signs/symptoms.
Developmental delays at arrival
It was standard practice for internationally adopted children to receive comprehensive medical examinations upon arriving in the adoptive country. In 2005, we asked parents to report whether their child was assessed to have no/minor delay (coded as 0), moderate delay (coded as 1), or severe delay (coded as 2) in four developmental domains: Gross Motor, Fine Motor, Social, and Cognitive skills. This was used as another indicator of preadoption adversity. A composite developmental delay score was created by averaging the item scores (ranging from 0 to 2).

Initial adaptation to adoption
Parents were presented with a list of 10 items generated from the same earlier study that facilitated the development of the above-mentioned measure of signs and symptoms. Items focused on the child’s early behavioral patterns in interactions with parents (e.g. avoiding affection) and in adjustment to new routines (e.g. eating, bathing, napping) during the first 3 weeks following adoption. Parents were first asked to check whether a given behavior was observed (yes or no) and if so, approximately how long it lasted (<1, 1–2, and ≥ 3 weeks). Responses were recoded as 0 (behavior not observed), 1 (observed for 2 weeks or less), and 2 (observed for ≥ 3 weeks). These behaviors were used to reflect the third area of preadoption adversity: adopted children’s preadoption social-emotional adversity. Exploratory factor analysis using principal axis factoring and promax rotation (Tan et al., 2007) extracted two factors that were conceptually and statistically meaningful: Avoidance/Refusal (α = 0.73; seven items, e.g. the child pulled away when held face-to-face by parent) and Crying/Clinging (α = 0.65; three items, e.g. crying when separated from parent). Summary scores were computed for each factor by averaging the item scores so that Avoidance/Refusal and Crying/Clinging were scaled from 0 (behavior not observed) to 2 (observed for 3 weeks or longer). In subsequent analyses, Avoidance/Refusal and Crying/Clinging were considered two separate indicators of preadoption adversity.

Concurrent family adversity
Informed by the study of Enoch et al., (2010), we considered the adopted children’s concurrent stressful experience in data analysis. We used measures of the adoptive mother’s authoritarian parenting behaviors and of problems experienced within the adoptive family (e.g. financial or marital difficulties) to reflect family adversity. Data were obtained in 2009 using the Parenting Styles and Dimensions Questionnaire (Robinson et al., 2001) and the Social Problems Questionnaire (Corney and Clare, 1985), respectively. We should note that the family problems scale was constructed to only include stresses that were not directly attributed to the behavior of the child. The internal consistency was adequate for both authoritarian parenting (α = 0.71) and family problems (α = 0.76). In the current analysis, the sum score of each scale was used.

Childhood behavioral problems
In 2009, when the children were 8.1 years on average (SD = 2.9), their adoptive mothers completed the Child Behavior Checklist (CBCL; Achenbach and Rescorla, 2000, 2001). The CBCL asked parents to rate the child’s behaviors (e.g. Defiant) as 0 (Not True of the child), 1 (Somewhat/Sometimes True), or 2 (Very True/Often True). The CBCL yielded two broadband summary scales: internalizing problems (e.g. depression) and externalizing problems (e.g. aggression). In our sample, the internal consistency was high for internalizing problems (α = 0.81) and externalizing problems (α = 0.89). As recommended by Achenbach and Rescorla (2001), we used standardized scores (T-scores) for data analysis.

MAOA genotyping
In 2010, when the adopted girls were 9.2 years old on average, we collected saliva samples from the adopted children using Oragene Kits (DNA Genotek, Ottawa, Ontario, Canada). For a subsample of 40 girls, saliva samples were collected again using the same method in 2011 to be analyzed by a different lab to cross-check the accuracy of the genotyping. Genotypes from the two labs matched completely.

Results
Descriptive statistics
The distribution of the 94 adopted Chinese girls’ MAOA genotypes was as follows: 2R/4R (n = 1, 1.06%), 3R/3R (n = 41, 43.6%), 3R/4R (n = 34, 36.2%), and 4R/4R (n = 18, 19.2%). These genotype frequencies were consistent with the Hardy–Weinberg equilibrium. For the purposes of this study, we focused on the 4R (purportedly a ‘high-activity’) allele. There was no difference in the demographics or measures of preadoption adversity among the adoptees with zero, one, or two of the 4R alleles.

Main effect of preadoption adversity
For data analysis, we followed the approach used by Enoch et al. (2010). We carried out a correlation analysis between the four indicators of preadoption adversity and the adopted Chinese girls’ internalizing and externalizing problems. None of the correlations were significant, suggesting that preadoption adversity did not have a main effect on these girls’ current behavior problems.

Main effect of MAOA
Again following the approach of Enoch et al. (2010), we compared the internalizing and externalizing problem scores of adopted Chinese girls who carried zero, one, or two of the 4R MAOA alleles. The results (Fig. 1) showed that for internalizing problems, those with no 4R allele (i.e. 0 4R, n = 41, M = 52.73, SD = 10.81) scored higher…

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than those with two 4R alleles at a nominal level of significance (i.e. 2 4R, \( n = 18 \), \( M = 46.44 \), SD = 8.60; \( t = 2.20, P < 0.05 \)), but did not score significantly higher than those with one 4R allele (i.e. 1 4R, \( n = 35 \), \( M = 50.60 \), SD = 9.94, \( t = 1.42, P = 0.16 \)). For externalizing problems, there was a nonsignificant tendency for children with no 4R allele (i.e. 0 4R, \( n = 41 \), \( M = 50.44 \), SD = 10.51) to score higher than those with two 4R alleles (i.e. 2 4R, \( n = 35 \), \( M = 49.60 \), SD = 9.55, \( t = 0.36, P = 0.72 \), or one 4R allele (i.e. 1 4R, \( n = 18 \), \( M = 47.56 \), SD = 11.17, \( t = 0.95, P = 0.35 \)). Overall, these results suggest that \( MAOA \) had a main effect on internalizing problems.

**Preadoption adversity and \( MAOA \) interaction**

Finally, we again followed the approach used by Enoch et al. (2010) to test whether there was any \( MAOA \times \) adversity interaction. For each outcome variable, we first ran a set of exploratory regression analyses to determine whether there was a significant interaction between any one of the adversity indicators and \( MAOA \). The following variables were entered into each analysis: \( MAOA \) (i.e. number of 4R alleles), each of the four adversity indicators, and an interaction term involving \( MAOA \times \) one of the adversity indicators. In addition, we included two demographic variables (i.e. child’s age and adoptive family household income) and two concurrent contextual variables (i.e. authoritarian parenting and family problems) to serve as covariates. A significant interaction was found only for the physical signs and symptoms measure, suggesting that \( MAOA \) had a moderating effect on the effect of signs/symptoms of pre-adoption adversity and the adopted children’s internalizing and externalizing problems. The results are summarized in Table 1.

As shown in Table 1, a significant effect (\( P < 0.01 \)) for authoritarian parenting was found for externalizing problems and a nominally significant effect (\( P < 0.051 \)) was found for internalizing problems. Greater authoritarian parenting was associated with higher scores for both internalizing and externalizing problems.

A nominally significant interaction (\( P < 0.05 \)) between \( MAOA \) and physical signs of adversity was also found for internalizing problems (Table 1). Post-hoc analyses showed that number of 4R alleles significantly differentiated the adopted Chinese girls’ internalizing problem scores when no signs of pre-adoption adversity were reported (the mean internalizing problem scores were 52.67, 47.04, and 41.41 for children with zero, one, or two 4R alleles, respectively; \( P = 0.0019 \)). The number of 4R \( MAOA \) alleles was not significant in differentiating the adopted Chinese girls’ internalizing problem scores when one physical sign of adversity was present (the mean internalizing problem scores were 52.82, 50.71, and 48.60 for carriers of zero, one, or two 4R alleles, respectively; \( P = 0.176 \)) or when two signs of pre-adoption adversity were reported (means were 52.98, 54.39, and 55.80; \( P = 0.600 \)).

The pattern of results suggested that a greater number of 4R alleles was related to lower internalizing problems for children who showed no physical signs of adversity at the time of adoption (Fig. 2), but was not associated with lower internalizing scores when one or more physical signs had been observed.

In addition, an interesting (albeit nonsignificant) interaction between \( MAOA \) and physical signs was found for externalizing problems (\( P = 0.085 \)). Again, a greater number of 4R alleles were related to lower externalizing

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**Table 1** Summary of the regression results on the effect of preadoption adversity measures and \( MAOA \) on Chinese girls’ internalizing and externalizing problem scores (\( N = 94 \))

|                      | Internalizing | Externalizing |
|----------------------|---------------|---------------|
| Intercept            | 38.37***      | 18.67*        |
| Covariates           |               |               |
| Age of the child     | -0.23         | 0.12          |
| Household income     | 0.00          | 0.09          |
| Authoritarian parenting | 4.74*     | 15.64***      |
| Family problems      | 7.14          | 4.56          |
| Preadoption adversity |              |               |
| Signs/symptoms of preadoption adversity | 0.15      | -1.79         |
| Developmental delays upon arrival | -2.63      | 1.97          |
| Crying/clingy behaviors during first 3 weeks | 0.41       | 1.12          |
| Refusal/avoidance behaviors during first 3 weeks | -3.09      | -4.52         |
| Number of 4R \( MAOA \) alleles | -5.63**     | -3.22         |
| \( G \times E \) interaction | 3.52*      | 2.67          |
| \( MAOA \times \) signs/symptoms of preadoption adversity | 2.00*      | 3.65**        |
| \( R^2 \) (%)        | 19.4          | 30.8          |

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Data on behavior problems were collected in 2009. Data on indicators of preadoption adversity were collected in 2005. \( \sim P < 0.10 \). \( *P < 0.05 \). \( **P < 0.01 \). \( ***P < 0.001 \).
problems for children who showed fewer physical signs of adversity at the time of adoption (Fig. 3). Post-hoc analyses showed that when no signs of adversity were reported, number of 4R alleles differentiated the adopted Chinese girls’ externalizing problems at a nominal level of significance (the means were 50.87, 47.65, and 44.43 for zero, one, and two 4R allele carriers, \( P = 0.051 \)). However, a number of 4R MAOA alleles did not differentiate the adopted Chinese girls’ externalizing problems when there was one sign reported (the means were 48.30, 47.75, and 47.20 for zero, one, and two 4R allele carriers, \( P = 0.702 \)) or two signs reported (the means were 46.50, 48.62, and 50.74 for zero, one, and two 4R allele carriers, \( P = 0.396 \)). This difference disappeared and was even slightly reversed as the number of physical signs increased.

**Discussion**

Adoption has been considered a useful natural experiment because of its potential for identifying the effects of early adversity that may persist into later childhood or adulthood. We investigated how the adopted Chinese girls’ internalizing and externalizing problems were predicted by indicators of their history of preadoption adversity, their MAOA genotype, and the interaction between preadoption adversity and MAOA. We also considered two indicators of postadoption adversity (authoritarian parenting, family problems) to avoid confounding preadoption and postadoption stressors. Consistent with a considerable body of past research on nonadopted children (see Maccoby and Martin, 1983; Hart et al., 2003), we found that authoritarian parenting by the adoptive family was related to both internalizing and externalizing problems. However, beyond the effects of parenting style, we obtained several findings that contribute toward our knowledge of genes, environment, and behavioral problems in internationally adopted children.

Most previous research on MAOA has focused on externalizing behavior in boys. Although a number of studies have found that the 4R variant of MAOA-VNTR is associated with fewer externalizing problems (e.g., Caspi et al., 2002; Kim-Cohen et al., 2006), one previous investigation of internationally adopted boys (Van der Vegt et al., 2009) obtained a contrasting result, with boys carrying the 4R allele showing more externalizing problems than boys with the 3R allele. Fewer studies of MAOA and externalizing problems have been carried out with girls. Although several of these have found that a higher number of 4R alleles is related to less behavior associated with externalizing problems (e.g., Prom-Wormley et al., 2009; Enoch et al., 2010), Aslund et al. (2011) found that maltreated girls with one or two ‘high-activity’ alleles (3.5R, 4R, or 5R) reported more antisocial behavior than girls with other variants of the polymorphism.

Our investigation extended previous research on MAOA and behavioral adjustment in adopted children by including measures of internalizing as well as externalizing problems. Our regression analyses found that (when controlling for several postadoption experience variables), adopted Chinese girls with a higher number of 4R alleles had significantly fewer internalizing problems than their peers with fewer 4R alleles. However, further analyses suggested that this difference was found only for the girls who had shown no observable physical signs of adversity at the time of their adoption (who constituted the largest subgroup in our study). As the 4R allele is often assumed to reflect high MAOA activity, our findings suggest that such high activity may function as a protective factor against the development of internalizing problems in girls who have suffered adversity invariably associated with early institutional care in Chinese orphanages, but that this protective effect may dissipate.
Several investigators (e.g. Caspi et al., 2007) have suggested that high-activity MAOA benefits maltreated children by counteracting the effects of severe maltreatment on their neurotransmitter systems. That is, maltreatment may cause these systems (particularly DA, 5HTT, and NE) to become hyper-reactive, a response that may be somewhat counteracted by the degradation of these transmitters by MAOA. However, some of our results along with those reported in the previous literature suggest that a more nuanced picture is required. In particular, our results suggest that high-activity MAOA is not protective with respect to moderately maltreated children who have experienced greater levels of preadoption adversity. Furthermore, although Cicchetti et al. (2007) found ‘high activity’ (defined as 3.5R, 4R, or 5R) to be protective with respect to severely maltreated children’s internalizing problems, they found the opposite patterns for moderately maltreated children (i.e. low-activity MAOA was protective). Thus, further research is required that focuses on the effects of MAOA on specific neurotransmitter systems developing in children experiencing different types and levels of adversity.

In contrast to previous studies of internationally adopted children, one strength of our investigation is that participants were identical in terms of sex and ethnicity. This partially offset the limitation of our small sample size. However, studying sex effects in adopted children from other cultures (i.e. in which both sexes are available for adoption in more equal numbers) would be especially important as MAOA is an X-linked gene and internalizing problems (such as depression) are less common in males than in females (Keenan and Hipwell, 2005). A second strength of our study was the fact that our measures of preadoption adversity and psychological adjustment were obtained 4 years apart. This partially offset the limitation of shared method variance (i.e. parent report for both measures) and backward reference (i.e. the influence of child’s current adjustment on parents’ reports of early adversity). Nonetheless, the small sample size presented some challenges in significance testing. We recommend that future studies include a larger sample size to allow for more stringent analyses to address potential problems with multiple testing.

Our study also had the challenge of the lack of direct assessment of preadoption adversity by observational measures within the institutions. Unfortunately, as noted above, it has not been possible to obtain direct measures of institutional deprivation in Chinese orphanages. However, persisting efforts might be made to do so in future studies. In addition, future research utilizing larger samples should also be carried out to confirm the results obtained in our study.

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