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# *RORA* Polymorphism Interacts with Childhood Maltreatment in Determining Anxiety Sensitivity by Sex: A Preliminary Study in Healthy Young Adults

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**Objective:** Recent studies have reported associations of retinoid-related orphan receptor alpha (RORA) gene single nucleotide polymorphisms (SNPs) with depression and anxiety disorders. Based on these, we attempt to test whether *RORA* polymorphism is associated with anxiety sensitivity (AS), the intermediate phenotype of depression and anxiety disorders. Considering gene-environment interactions and sex differences in AS, childhood maltreatment (CM) and sex were considered as confounders.
**Methods:** Two-hundred and five healthy young Korean adults (female: 98, male: 107; age, 23.0±3.2 years) completed genotyping for the *RORA* SNP rs11071547, as well as measures for AS and CM. Generalized linear models were used to examine the main and interaction effects of *RORA* genotype, CM, and sex in determining AS.
**Results:** The main effect of *RORA* polymorphisms was not found ($p$=0.760) whereas the main effect of CM and interaction effects among sex, genotype, and maltreatment were significant on AS. In separate analyses by sex, the interaction effect between *RORA* genotype and maltreatment was significant only in males ($p$<0.001). In females, the main effects of genotype and CM were significant (both were $p$<0.001), in which both a history of CM and C genotype tended to be associated with higher AS.
**Conclusion:** The association between RORA polymorphism and AS might differ by sex. The interaction between RORA polymorphism and CM was significant only in males whereas *RORA* genotype and CM independently associated with AS in females. Further studies are encouraged to confirm the relationship between RORA polymorphism and AS.

KEY WORDS: Gene-environment interaction; Retinoid acid receptor-related orphan receptor alpha (*RORA*) gene; Childhood trauma; Sex; Anxiety sensitivity.

## INTRODUCTION

It has been suggested that retinoid-related orphan receptor alpha (RORA) gene single nucleotide polymorphisms (SNPs) pose a risk for depression[1-3] and fear-related internalizing psychopathologies including panic, phobia, and obsessive-compulsive disorders.[4] Recent reports have also demonstrated that *RORA* polymorphisms might be associated with posttraumatic stress disorder after exposure to various types of lifetime trauma[5] and a hurricane.[6] These results put questions whether *RORA* polymorphism is associated with an intermediate phenotype of depression and anxiety disorders, as well as how it interacts with traumatic experiences.

Anxiety sensitivity (AS) has been proposed as the potential intermediate phenotype of depression and anxiety disorders.[7,8] The individual levels of AS are thought to be influenced by genetic and environmental factors, such as childhood maltreatment (CM).[9,10] Regarding genetic factors, the polymorphisms of serotonin transporter gene[7,11] and neuropeptide S receptor gene[12] have been reported to interact with CM in determining AS.

We aimed to test whether the *RORA* genotype is associated with AS among healthy young adults. Considering the proposed sex differences in AS[13] as well as the gene-environment interaction in determining AS,[14,15] CM and sex were regarded as possible confounders.

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## METHODS

### Participants

Healthy young adults were recruited by newspaper advertisement in Seoul, Republic of Korea. Participants were confirmed to be free from lifetime or current diagnosis of any psychiatric disorders and neurologic diseases through an interview with an experienced psychiatrist using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (SCID I and II). Among the 205 unrelated Koreans completed study procedures, 107 (52.2%) were male and a mean age was 23.0 (standard deviation [SD], 3.2; range, 18-31) years. All participants provided written informed consent prior to participation. The study protocol was approved by the Institutional Review Board at Seoul St. Mary's Hospital, The Catholic University of Korea (KC10TIMI0434).

### Measures

Individual AS was measured by Anxiety Sensitivity Index-Revised (ASI-R).[16,17] It is rated on a 5-point Likert scale from 0 to 4. Higher total score means higher AS.

The short form of the Childhood Trauma Questionnaire (CTQ) was used to evaluate a history of CM.[18,19] The CTQ is a self-report questionnaire consisting of 28 items that measure five categories of CM including emotional and physical neglect as well as emotional, physical, and sexual abuse. Each category consists of five items rated on a 5-point Likert scale. Based on the manual specifying cutoff points for the levels of none, low, moderate, and severe,[20] the moderate to severe cutoff scores for each subscale were used to determine positive in that category and having one or more positive categories was regarded as the presence of CM.[21,22]

### Genotyping

The *RORA* rs11071547 was selected based on the previous study that reported its association with sleep disorder as one of the circadian clock genes.[23] The rs11071547 is located in the intron region of *RORA* gene on chromosome 15. Blood samples (5-10 ml) were collected into an ethylenediaminetetraacetic acid (EDTA) tube, and genomic DNA was isolated using a NucleoSpin® Blood DNA Extraction Kit (Macherey-Nagel, Düren, Germany) according to procedures from the manufacturer's manual. Genotyping was performed by high-resolution melting curve analysis. Polymerase chain reaction (PCR) was performed in a volume of 20 μl per reaction with a 96-well Bio-Rad CFX96 Real Time PCR system (Bio-Rad, Hercules, CA, USA). Reaction mixtures included 1.5 μl of genomic DNA as a template and each primer for the rs11071547 SNP of *RORA* at 200 mM (forward 5'-TGC CTA CCG CTT TCC TTT-3', and reverse 5'-AAA TAA ACT TGG AGT GTT CTG GA-3'), 1×Sso Fast EvaGreen SuperMix (Bio-Rad) and sterile $H_2O$. The amplification protocol started with 98°C/3 minutes followed by 39 cycles of 98°C/10 seconds and 58°C/20 seconds. After an initial step of 95°C/10 seconds and 65°C/10 seconds, melting curves were generated from 65°C to 95°C in increments of 0.3°C/cycle. Melting profiles were analyzed with Bio-Rad Precision Melt software.

### Statistics

The Hardy-Weinberg equilibrium for genotype distribution was tested by the $\chi^2$ test. Between-group comparisons were performed with independent *t*-test or one-way analysis of variance and $\chi^2$ test for continuous and categorical variables, respectively. The relationship between continuous variables was examined by Pearson's correlation analysis. Generalized linear models were used to explore the main effects of sex, *RORA* genotype, and CM with all possible two- and three-way interaction effects on AS. Data were analyzed with SAS software ver. 9.0 (SAS Institute, Inc., Cary, NC, USA). All statistical tests were two-tailed, and $\alpha$ was set at 0.05.

## RESULTS

Distributions of the alleles and genotypes of RORA rs11071547 were in Hardy-Weinberg equilibrium ($\chi^2$=0.480, $p$=0.488). Among 205 participants, the allelic frequencies of C and T were 36.1% (n=148) and 63.9% (n=262), respectively. These frequencies are similar to reported distributions among general populations of China and Japan in the HapMap database (http://www.hapmap.org). The genotype frequencies of C/C, C/T, and T/T were 14.1% (n=29), 43.9% (n=90), and 42.0% (n=86), respectively. We reclassified the three genotypes into two groups, C (C/C and C/T, n=119) and T/T (n=86).

Mean scores of AS was 20.8 (SD=14.8). One or more types of CM were reported among 87 (42.4%) participants, in which 32, 30, 11, 54, and 24 individuals reported a history of emotional neglect, physical neglect, emotional abuse, physical abuse, and sexual abuse, respectively. Participants with CM showed significantly higher AS than those without ($p$=0.015). The frequencies of *RORA* genotype and sex as well as the mean ages did not

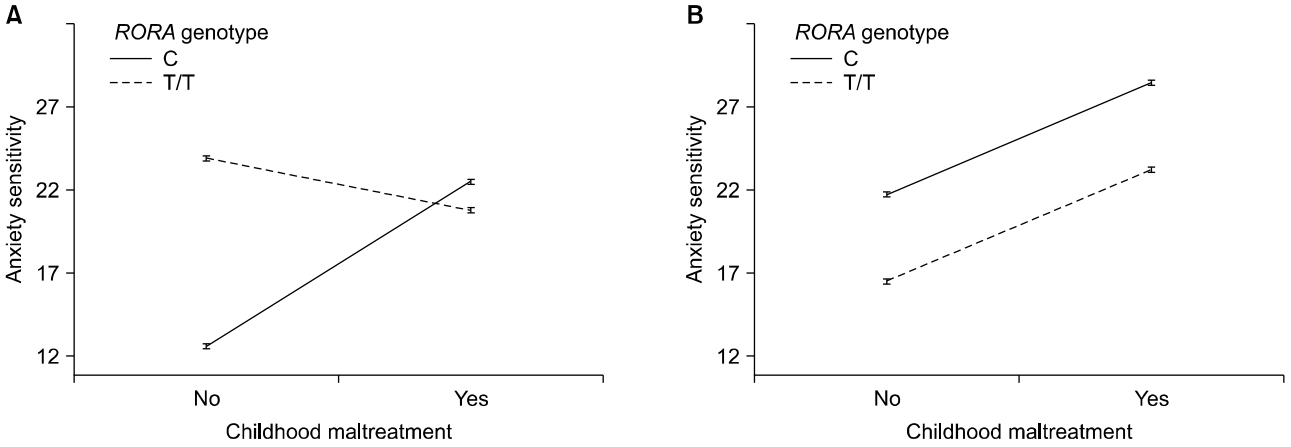


**Fig. 1.** Interaction effects between *RORA* polymorphism and childhood maltreatment on anxiety sensitivity by sex. The interaction effect was significant in male subects (A), whereas main effects of RORA genotype and childhood maltreatment were significant in female subjects (B).

**Table 1.** Results of generalized linear model examining the effect of sex, childhood maltreatment, and *RORA* genotype on anxiety sensitivity (WALD type 3 statistic)

| Source | df | $\chi^2$ test | *p* value |
|---|---|---|---|
| Age | 1 | 83.02 | <0.001 |
| Sex | 1 | 13.36 | <0.001 |
| Genotype (*RORA* polymorphism) | 1 | 0.09 | 0.760 |
| Maltreatment | 1 | 58.13 | <0.001 |
| Sex×genotype | 1 | 59.46 | <0.001 |
| Sex×maltreatment | 1 | 2.61 | 0.106 |
| Genotype×maltreatment | 1 | 20.49 | <0.001 |
| Sex×genotype×maltreatment | 1 | 34.43 | <0.001 |

Omnibus test ($p<0.001$).
df, degree of freedom.

differ significantly by a history of CM ($p$=0.668, 0.114, 0.791, respectively). Age showed significant negative association with AS (r=−0.178, $p$=0.010).

In the generalized linear model controlling for age (Table 1), the main effect of *RORA* genotype was not significant whereas that of CM was significant in predicting AS. There were significant three-way interaction among sex, genotype, and CM. In separate models by sex (Fig. 1), the interaction effect between genotype and CM was significant only in males ($\chi^2$=64.70, $p<0.001$). The T/T genotype was associated with the higher AS than C genotype among males without CM ($p$=0.016), whereas there was no difference in AS by genotype among males with CM ($p$=0.565). In females, the main effects of genotype and CM were significant ($\chi^2$=19.73, $p<0.001$ and $\chi^2$=27.25, $p<$ 0.001, respectively), in which the history of CM and C genotype were associated with higher AS.

## DISCUSSION

This preliminary study did not found the association between *RORA* rs11071547 genotype and AS. However, we demonstrated the sex-specific interactions between *RORA* genotype and CM in determining AS. The *RORA* genotype interacted with CM in males, whereas each of *RORA* genotype and CM were significant predictor of AS in females. These results suggest the possible role of sex in interaction between *RORA* polymorphism and CM to determine AS.

RORA protein plays a role in maintaining circadian rhythm.[24] Clinical studies have shown that disruptions in circadian rhythms may contribute to anxiety-prone traits and/or anxiety disorders.[25] Among circadian-clock genes, the SNPs of *ARNTL2*, *DRD2*, *BCL2*, and *PAWR* have been reported to be associated with AS.[26,27] In addition, RORA protein is widely expressed in brain regions including the frontal cortex,[28,29] and participates in protecting neurons and glial cells from oxidative stress-induced apoptosis.[30] Oxidative stress is one of the potential mechanisms implicated in pathophysiology of depression and anxiety disorders. In animal studies, high levels of oxidative stress induced anxiogenic behaviors[31] and vulnerability to depression.[32] These roles of RORA protein might support the biological plausibility of the association between *RORA* polymorphism and AS.

Results of the present study emphasize the importance of the sex in gene-environment interaction in determining AS. As in previous studies,[9,10] CM was a significant environmental factor influencing AS. However, we found that CM interacts with *RORA* genotype in males but not in females. Among females, the main effects of genotype

and maltreatment were significant. These results suggested that the level AS might be influenced differently to CM according to *RORA* genotype in males whereas it might be expected by the genotype and the experience of CM in females. These results are partly in line with that of a twin study, in which AS is heritable only in women and better explained by environmental factors in men.[33] It has also been reported that heritability and the impact of genetic factors might be greater among females than those among males in the studies of anxiety disorder and neuroticism.[14,15] Regarding the behavioral impact of circadian genes, a the *CLOCK* gene mutation has been reported to induce more pronounced behavioral changes of increased exploratory behaviors in female relative to male mice.[34] Based on the results of previous and present studies, we could hypothesize that the impact of related genes including circadian genes might be differently influenced by environmental factor on AS by sex. Further studies on the effect of sex hormones and sexual difference in epigenetic processes on brain and behaviors[35] would be required to attest our hypothesis.

There are several limitations. Sample size is small and the study design is cross-sectional. A retrospective assessment of CM may be biased by individual levels of emotionality[36] though recall bias has been reported to account for less than 1% of variance in non-clinical young adults.[37] In addition, the clinical significance of *RORA* rs11071547 is yet to be determined and we did not analyze other *RORA* SNPs, which have been reported the associations with depression and anxiety disorders.[4-6] Recently suggested critical issues of G×E approach need to be duly considered.[38]

In summary, we did not found the association between *RORA* rs11071547 and AS among healthy young adults. However, we provide the evidence of the sex-specific interaction between *RORA* polymorphism and CM in determining AS.

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