The role of stress-regulation genes in moderating the association of stress and daily-life psychotic experiences

Cristóbal-Narváez P, Sheinbaum T, Myin-Germeys I, Kwapil TR, de Castro-Catala M, Domínguez-Martínez T, Racioppi A, Monsonet M, Hinojosa-Marqués L, van Winkel R, Rosa A, Barrantes-Vidal N. The role of stress-regulation genes in moderating the association of stress and daily-life psychotic experiences.

Objective: The interaction of single nucleotide polymorphisms with both distal and proximal environmental factors across the extended psychosis phenotype is understudied. This study examined (i) the interaction of relevant SNPs with both early-life adversity and proximal (momentary) stress on psychotic experiences (PEs) in an extended psychosis sample; and (ii) differences between early-psychosis and non-clinical groups for these interactions.

Methods: Two hundred and forty-two non-clinical and 96 early-psychosis participants were prompted randomly eight times daily for 1 week to complete assessments of current experiences, including PEs and stress. Participants also reported on childhood trauma and were genotyped for 10 SNPs on COMT, RGS4, BDNF, FKBP5, and OXTR genes.

Results: Unlike genetic variants, distal and proximal stressors were associated with PEs in both samples and were more strongly associated with PEs in the early-psychosis than in the non-clinical group. The RGS4 TA and FKBP5 CATT haplotypes interacted with distal stress, whereas the A allele of OXTR (rs2254298) interacted with proximal stress, increasing momentary levels of PEs in the early-psychosis group. No interactions emerged with COMT or BDNF variants.

Conclusion: Individual differences in relevant stress-regulation systems interact with both distal and proximal psychosocial stressors in shaping the daily-life manifestation of PEs across the psychosis continuum.
Significant outcomes

- Stress-sensitivity mechanisms seemed to be shared across non-clinical and clinical levels of the hypothetical continuum of psychosis.
- Complex gene–environment interactions emerged on the daily-life expression of psychotic phenomena in help-seeking individuals compared with non-clinical individuals.

Limitations

- The cross-sectional nature of the ESM data limits interpretations about causation.
- The sample composition precludes definite conclusions about the role of schizotypy in the GxE interactions.

Introduction

Converging evidence suggests that the psychosis phenotype is expressed across a dynamic continuum spanning from subclinical (e.g., schizotypy, psychotic-like experiences) to full-blown psychotic manifestations (1, 2). In recent years, increasing focus has been placed on studying persons at the early stages of psychosis, such as those with at-risk mental states for psychosis (ARMS) and first episode psychosis (FEP). These populations allow us to examine potentially etiologically relevant mechanisms of psychotic disorders without the marked confounding factors seen in chronic patients (3). Notably, several studies report an overlap of etiological factors, as well as phenomenological and developmental processes, across high schizotypy, clinical risk, and clinical populations (e.g., 4, 5).

Among these psychosocial factors, both distal (early-life adversity) and proximal (daily-life momentary) stress have been associated with psychotic features across the extended psychosis phenotype (5–8). At the same time, and consistent with the hypothesized etiopathogenic relevance of these factors, psychosis populations have higher levels of trauma exposure and stress sensitivity (9).

Gene–environment interaction research (GxE) also highlights the synergistic effect between environmental and genetic risk factors across subclinical and clinical expressions of psychosis (4, 10). In this sense, a limited but increasing number of GxE studies have shown that certain single nucleotide polymorphisms (SNPs) interact with distal or proximal stress to heighten risk for psychotic experiences (PEs; e.g., 11). For example, it has been shown that the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism moderates the psychosis-inducing effects of distal (12, 13) and momentary stress (14). However, scarce research has examined the interaction of genetic variants with both distal and proximal stress within the same study. Such an approach should offer valuable insights, especially considering recent work indicating that childhood and adult stress interact differently with genetic variants linked to hypothalamus-pituitary-adrenal (HPA) axis reactivity in stress-related phenotypes (15). In particular, previous research showed that some SNPs (e.g., rs3800373, rs9296158, rs1360870, and rs9470080) on the FK506 binding protein 5 (FKBP5) gene interacts with distal, but not proximal, stress in the expression of PEs (16–19).

Another important consideration is that only a few studies have employed experience sampling method (ESM) to examine GxE interactions in the realm of daily life, which offers the advantage of minimizing retrospective bias and enhancing ecological validity (20, 21). ESM studies have predominantly examined the interaction of momentary stress with genetic variation in dopamine-related genes, such as the catechol-O-methyltransferase (COMT) on the emergence of PEs (22). However, differential effects of COMT Val158Met alleles have been found in non-clinical and clinical samples (14, 22–24). Other candidate genes related with dopamine signaling that have been associated previously with PEs, such as the regulator of G-protein signaling 4 (25–27), are also of interest for exploring their possible involvement in the development of such experiences through a stress-reactivity pathway. The oxytocin receptor gene (OXTR) is another promising candidate for understanding individual differences in the response of the dopaminergic and stress-response systems. Two OXTR SNPs (rs53576, rs2254298) have been identified as relevant in the context of mental disorders associated with social deficits (28). Crucially, the interconnections between the dopamine and oxytocin systems have led to the suggestion that individuals with more efficient variants (i.e., the G alleles of both OXTR SNPs) may regulate more adaptively the salience assigned to social stimuli (29), thus diminishing susceptibility to psychopathology. Nonetheless, the role of OXTR variability in the context of stress reactivity in psychosis remains unexplored.
Aims of the study

The first aim was to concurrently examine the interaction of both distal (childhood trauma) and proximal (momentary) stress with genetic variation on psychotic experiences (PEs). The second aim was to examine whether the interaction of early-life adversity or real-life assessments of momentary stress (situational and social stress) with genetic variation on PEs differed between early-psychosis and non-clinical groups.

We predicted that the interaction of both distal and proximal environmental factors with the variability of \textit{COMT} (Val158Met Met allele), \textit{RGS4} (rs951436—rs2661319 TA haplotype), \textit{BDNF} (Val66Met Met allele), \textit{FKBP5} (rs3800373—rs9296158—rs1360780—rs4470080 CATT haplotype), and \textit{OXTR} genes (rs2554298 A allele and rs53576 A allele) would be associated with increased PEs and that these associations would be greater in an early-psychosis sample than in a non-clinical sample, given previous reports of increased levels of trauma exposure and stress sensitivity in persons with psychosis.

Methods

Participants

The data were collected as part of an ongoing longitudinal investigation examining psychosis risk and resilience. The non-clinical sample was drawn from an original unselected sample of 808 young adults, which included 547 undergraduate students from the Universitat Autònoma de Barcelona (UAB) and 261 students from technical training schools in Barcelona. A subset of these participants was invited to take part in an in-depth assessment including self-report, interview, laboratory, and ESM measures. We successfully recruited 136 participants who had standard scores based upon sample norms of at least 1.0 on the positive or negative schizotypy dimensions of the Wisconsin Schizotypy Scales (WSS; 30–33), the suspiciousness scale of the Schizotypal Personality Questionnaire (SPQ; 34), or the positive symptom subscale of the Community Assessment of Psychic Experiences (CAPE; 35), and randomly selected 106 participants who had standard scores below 1.0 on each of these. The goal of this enrichment procedure was to insure adequate variability of schizotypy traits and avoid having a ‘super healthy’ control sample. The final non-clinical sample comprised 242 participants (206 from UAB and 36 from technical schools). None of the university or technical school participant had a psychotic disorder according to SCID-I, and only eight participants met diagnostic criteria for Cluster A disorders: three with Schizotypal, three with Paranoid, and one with Schizoid personality disorder (one qualified for more than one disorder).

The early-psychosis sample was recruited in the Sant Pere Claver-Early Psychosis Program (SPC-EPP; 36) in Barcelona. A total of 96 early-psychosis participants (60 ARMS and 36 FEP) were included in this study. Patients’ inclusion criteria were age between 14 and 40 years old and IQ ≥ 75. ARMS criteria were established by the Comprehensive Assessment of At-Risk Mental States (CAARMS; 37) and/or the Schizophrenia Proneness Instrument-Adult version (SPI-A; 38). FEP patients met DSM-IV-TR criteria for any psychotic disorder or affective disorder with psychotic symptoms as established by the Structured Clinical Interview for DSM-IV (SCID-I; 39). All participants had full capacity to consent to participation in research and provided written informed consent prior to taking part in the study. The study was developed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethical approval for the study was granted by the university and the local ethics committees. Descriptive characteristics of the whole sample are displayed in supplementary material (Table S1).

Material and procedure

Distal stress

Participants were administered the Childhood Trauma Questionnaire (CTQ; 40), a self-report measure that assesses emotional, physical, and sexual abuse and emotional and physical neglect during childhood and adolescence. CTQ items are answered on a 5-point Likert-type scale ranging from ‘never true’ to ‘very often true’. In this study, the total CTQ score was used for analyses (Cronbach’s $\alpha = 0.89$).

ESM proximal stress and PEs measures

Experience sampling method data were collected on personal digital assistants (PDAs) that signaled participants randomly eight times daily (between 10 a.m. and 10 p.m.) for 1 week to complete brief questionnaires. All ESM items reported in the current study were answered on 7-point Likert-type scale ranging from ‘not at all’ to ‘very much’. The analyses used ESM items assessing PEs and appraisals of proximal (situational and social) stress. We created an index of PEs using the following 10 items: feeling suspicious, feeling mistreated, unusual senses, unusual thoughts,
Genetic data

All subjects were asked to provide a biological sample consisting of buccal mucosa on cotton swabs or blood. Genomic DNA was extracted using the Realpure genomic DNA extraction kit for buccal mucosa or blood samples (Durviz S.L.U., Valencia, Spain). Ten SNPs within the COMT, BDNF, OXTR, FKBP5, and RGS4 genes were genotyped using TaqMan 5' exonuclease assay (Applied Biosystems). Details on the SNPs are given in Table S2. Compliance with Hardy–Weinberg equilibrium was assessed for each SNP (all $P > 0.05$).

Linkage disequilibrium (LD) between SNPs within the same gene was examined by pairwise comparisons of $r^2$ and $D'$ using Haplovie version 4.2 (41). High LD was observed between the four FKBP5 SNPs and between the two RGS4 SNPs (both with $r^2 > 0.7$ and $D' > 0.9$), but not between the OXTR SNPs ($r^2 < 0.04$; $D' < 0.6$). Estimation of FKBP5 and RGS4 haplotype combination per subject was conducted using a Bayesian approach implemented with PHASE software (42). To better examine our hypotheses, participants were divided into the following groups based on previous studies (19, 26, 27, 43): (i) carriers of at least one risk haplotype, (ii) carriers of one risk haplotype and one protective haplotype, and (iii) carriers of at least one protective haplotype. Specifically, the groups were as follows: (i) AGCC/-, (ii) AGCC/CATT, and (iii) CATT/- for the FKBP5 haplotype, and (i) TA/-, (ii) TA/GG, and (iii) GG/- for the RGS4 haplotype. Haplotype frequencies are presented in Table S2.

Statistical method

Descriptive statistics were performed on the childhood trauma and ESM variables using the Statistical Package for Social Sciences (SPSS) Version 19.0 (44). ESM data have a hierarchical structure in which repeated daily-life ratings (level 1 data) are nested within participants (level 2 data). Linear mixed models were used to control for within-subject clustering of multiple observations using the ‘xtmixed’ command in Stata 12 (45). Graphs were generated with the R program (www.r-project.org). Analyses were performed on the total pool of participants, that is, on a total sample comprising non-clinical and early-psychosis participants, treating group as a variable when necessary.

The multilevel analyses examined two types of relations between genetic and environmental variables on PEs across the extended psychosis phenotype. First, to examine whether the interactions between environmental (distal and proximal stress) and genetic (four SNPs and two haplotypes) variables on PEs were significant in the total sample, the main effects of environmental and genetic variables (e.g., distal stress and FKBP5 haplotype) were entered simultaneously at the first step, and the interaction term (e.g., distal stress x FKBP5 haplotype) was entered at the second step to examine its contribution over and above the main effects.

Second, we examined whether the interactions between environmental and genetic variables on PEs differ between non-clinical and early-psychosis groups. Therefore, the three main effects (e.g., distal stress, FKBP5 haplotype, and group variables) were entered at the first step, the three-two-way interaction terms (e.g., distal stress x group, FKBP5 haplotype x group, and distal stress x FKBP5 haplotype) were entered at the second step, and the three-way interaction term was entered at the third step (e.g., distal stress x FKBP5 haplotype x group). When a significant interaction was found, the effect of the interaction was examined using simple slopes analyses. Distal and proximal stresses were used as continuous variables for analyses. Genotypes were coded 0, 1, and 2 using an additive genetic model. However, when genotype comparison was required, we also used a dummy variable coding. Six sets of multilevel models, one for each genetic variant investigated, were conducted to test each hypothesis; therefore, the $P$-value was set at $P = 0.05/6 = 0.0083$.

Results

GxE interactions in the total sample

As shown in Table 1, distal stress and both situational and social proximal stress were associated with PEs in daily life in the total sample, whereas
no main effects of genetic variation on PEs were found. The two-way interactions between genetic and environmental variables on PEs indicated that only the interaction of distal stress with the FKB5 (P = 0.007) and RGS4 (P = 0.008) risk haplotypes were associated with increased PEs. As expected, simple slopes analyses indicated that distal stress was associated with greater increases in PEs for individuals carrying the FKB5 risk haplotype (CATT/-: 0.028, SE = 0.008, P < 0.001; AGCC/CATT: 0.022, SE = 0.005, P < 0.001; AGCC/-: 0.010, SE = 0.003, P < 0.001). Additionally, dummy-coded variables were created for genotype comparison purposes. Participants carrying the FKB5 risk haplotype (CATT/- and AGCC/CATT) showed greater increases in PEs compared to non-risk individuals (CATT/- vs. AGCC/-: 0.018, SE = 0.007, P = 0.011; AGCC/CATT vs. AGCC/-: 0.012, SE = 0.006, P = 0.035; CATT/- vs. AGCC/CATT: 0.006, SE = 0.007, P = 0.402). Similarly, simple slopes analyses indicated that distal stress was associated with greater increases in PEs for individuals carrying the RGS4 risk haplotype (TA/-: 0.029, SE = 0.005, P = 0.000; TA/GG: 0.017, SE = 0.002, P = 0.000; GG/-: 0.015, SE = 0.004, P = 0.000). Participants carrying the risk haplotype (TA/-) experienced more PEs than TA/GG carriers (TA/- vs. TA/GG: 0.020, SE = 0.006, P < 0.001) and, at a trend level, than non-risk individuals (TA/- vs. GG/-: 0.014, SE = 0.007, P = 0.057). No differences were found between TA/GG and GG/- haplotype groups (TA/GG vs. GG: −0.006, SE = 0.007, P = 0.416).

Group differences in the interaction between environmental (distal and proximal) and genetic variables

As shown in Tables 2 and 3, the group variable was also associated with PEs, indicating that early-psychosis participants reported more PEs in daily life than non-clinical participants. The two-way interactions between environmental and group variables showed that, in most models (except for COMT and RGS4), environmental variables (both distal and proximal) were more strongly associated with PEs in the early-psychosis group. None of the two-way interactions of genetic variation with group were associated with PEs.

The three-way interaction of distal stress, FKB5 haplotype, and group was significantly associated with PEs, such that childhood trauma was associated with increased PEs for participants with the FKB5 risk haplotype in the early-psychosis group. Simple slope analyses indicated that the FKB5 risk haplotype moderated the association between distal stress and PEs in the early-psychosis, but not in the non-clinical group (early-psychosis: 0.024, SE = 0.09, P = 0.007; non-clinical: −0.004, SE = 0.002, P = 0.114). In addition, analyses of the FKB5 haplotype in the early-psychosis group showed that distal stress was associated with increased PEs for CATT/- and AGCC/CATT participants (CATT/-: 0.057, SE = 0.019, P = 0.002; AGCC/CATT: 0.029, SE = 0.014, P = 0.044), but not for those carrying the AGCC/- haplotype (AGCC/-: 0.003, SE = 0.006, ns; see Fig. 1).

---

Table 1. Two-way interactions of environmental stress variables and genetic variables on psychotic experiences

| Psychotic experiences | COMT rs4810 | BDNF rs6549 | OXTR rs2549236 | OXTR rs53576 | FKB5 Haplotype | RGS4 Haplotype |
|-----------------------|------------|-------------|----------------|--------------|----------------|---------------|
| Distal variable       |            |             |                |              |                |               |
| n = 316               | n = 318    | n = 319     | n = 309        | n = 319      | n = 315        |
| Coefficient (SE)               | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) |
| Childhood trauma       | 0.016 (0.002)*** | 0.018 (0.003)*** | 0.018 (0.003)*** | 0.018 (0.003)*** | 0.018 (0.003)*** | 0.018 (0.003)*** |
| Genetic variation (G)   | 0.008 (0.034) | 0.057 (0.043) | 0.020 (0.044) | −0.031 (0.037) | 0.010 (0.035) | 0.029 (0.032) |
| Childhood trauma x G    | −0.005 (0.004) | −0.008 (0.005) | −0.003 (0.006) | −0.004 (0.004) | 0.009 (0.003)* | 0.009 (0.004)* |
| Proximal variables      |            |             |                |              |                |               |
| n = 325               | n = 328    | n = 329     | n = 319        | n = 319      | n = 325        |
| Coefficient (SE)               | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) | Coefficient (SE) |
| Situational stress       | 0.054 (0.049)*** | 0.053 (0.005)*** | 0.055 (0.005)*** | 0.053 (0.005)*** | 0.056 (0.005)*** | 0.055 (0.005)*** |
| Genetic variation (G)   | 0.006 (0.024) | 0.023 (0.034) | −0.005 (0.034) | −0.051 (0.029) | 0.023 (0.027) | 0.029 (0.025) |
| Situational stress x G    | 0.001 (0.007) | 0.001 (0.008) | 0.015 (0.009) | 0.002 (0.007) | 0.007 (0.007) | −0.001 (0.006) |
| Close to other          | 0.003 (0.030) | 0.013 (0.043) | 0.015 (0.043) | 0.033 (0.037) | 0.038 (0.035) | 0.072 (0.032) |
| Prefer to be alone      | 0.034 (0.006)*** | 0.032 (0.005)*** | 0.035 (0.006)*** | 0.034 (0.005)*** | 0.036 (0.006)*** | 0.036 (0.006)*** |
| Genetic variation (G)   | −0.023 (0.023) | 0.056 (0.053) | 0.005 (0.033) | 0.002 (0.010) | 0.003 (0.008) | 0.006 (0.008) |
| Prefer to be alone x G    | 0.007 (0.008) | −0.017 (0.009) | 0.002 (0.010) | 0.003 (0.008) | 0.005 (0.008) | 0.005 (0.008) |

*P < 0.01, ***P < 0.001. In order to examine the two-way interaction between genetic and environmental variables, we first assessed the two main effects of E and G in the same model and then entered the interaction term over and above the two main effects.
Similarly, the three-way interaction among distal stress, *RGS4* haplotype, and group was significantly associated with PEs. This indicated that distal stress was associated with increased PEs for participants with the *RGS4* risk haplotype in the early-psychosis group compared to the non-clinical group. Simple slopes analyses indicated a consistent trend for the early-psychosis group although it did not reach statistical significance (early-psychosis: $0.017$, $SE = 0.010$, ns; non-clinical: $-0.003$, $SE = 0.003$, $P = 0.204$). Additionally, analyses of the *RGS4* haplotype in the early-psychosis group showed that distal stress was associated with increased PEs for the risk haplotype TA/- participants (0.034, $SE = 0.009$, $P < 0.001$), but not for those carrying the GG/- or TA/GG haplotype (GG/-: 0.750, $SE = 0.929$, $P = 0.42$; TA/GG: 1.170, $SE = 0.161$, $P = 0.055$).

The three-way interactions also examined whether the interaction of proximal stress (both

---

### Table 2. Three-way interactions of group status (early-psychosis vs. non-clinical), environmental (distal and proximal) stress variables, and genetic variables on psychotic experiences

| Psychosocial stress | COMT rs4680 | BDNF rs5285 | OXTR rs2254298 | OXTR rs53576 | FKBP5 Haplotype | RGS4 Haplotype |
|---------------------|-------------|-------------|----------------|-------------|-----------------|---------------|
| **Distal variable** | n = 316     | n = 319     | n = 319        | n = 309     | n = 309         | n = 315       |
| Childhood trauma   | $0.012 (0.002)**$ | $0.013 (0.002)**$ | $0.015 (0.002)**$ | $0.014 (0.003)**$ | $0.013 (0.003)**$ | $0.013 (0.003)**$
| Genetic variation (G) | $0.014 (0.032)$ | $0.055 (0.040)$ | $0.012 (0.041)$ | $-0.018 (0.036)$ | $0.008 (0.033)$ | $0.024 (0.030)$
| Group               | $0.343 (0.056)**$ | $0.345 (0.056)**$ | $0.360 (0.057)**$ | $0.350 (0.057)**$ | $0.365 (0.058)**$ | $0.358 (0.057)**$
| Childhood trauma x G | $0.011 (0.005)$ | $0.015 (0.005)$ | $0.014 (0.005)**$ | $0.015 (0.005)**$ | $0.014 (0.005)**$ | $0.011 (0.005)$
| G x group           | $-0.006 (0.080)$ | $0.242 (0.086)$ | $0.021 (0.095)$ | $-0.075 (0.083)$ | $0.021 (0.079)$ | $0.121 (0.074)$
| Childhood trauma x G | $0.002 (0.004)$ | $-0.010 (0.005)$ | $-0.002 (0.004)$ | $0.002 (0.004)$ | $0.008 (0.003)+$ | $0.005 (0.004)$
| Childhood trauma x G x group | $-0.001 (0.009)$ | $-0.020 (0.010)$ | $-0.009 (0.009)$ | $0.003 (0.008)$ | $0.030 (0.006)**$ | $0.023 (0.007)**$
| **Proximal variables** | n = 325     | n = 328     | n = 329        | n = 319     | n = 319         | n = 325       |
| Situational stress | $0.054 (0.005)**$ | $0.053 (0.004)**$ | $0.055 (0.005)**$ | $0.054 (0.005)**$ | $0.056 (0.005)**$ | $0.056 (0.005)**$
| Genetic variation (G) | $0.010 (0.023)$ | $0.033 (0.032)$ | $-0.008 (0.032)$ | $-0.038 (0.028)$ | $0.023 (0.026)$ | $0.022 (0.024)$
| Group               | $0.202 (0.039)**$ | $0.250 (0.041)**$ | $0.247 (0.041)**$ | $0.245 (0.040)**$ | $0.248 (0.042)**$ | $0.245 (0.041)**$
| Situational stress x G | $0.075 (0.111)**$ | $0.068 (0.101)**$ | $0.073 (0.109)**$ | $0.069 (0.108)**$ | $0.074 (0.111)**$ | $0.073 (0.109)**$
| G x group           | $0.018 (0.054)$ | $0.097 (0.076)$ | $-0.039 (0.072)$ | $-0.122 (0.062)+$ | $0.116 (0.058)+$ | $0.110 (0.054)+$
| Situational stress x G | $0.003 (0.007)$ | $0.003 (0.008)$ | $0.013 (0.008)$ | $0.006 (0.007)$ | $0.007 (0.007)$ | $-0.002 (0.006)$
| Situational Stress x G x group | $0.008 (0.015)$ | $0.019 (0.018)$ | $0.051 (0.018)**$ | $0.004 (0.015)$ | $0.018 (0.015)$ | $0.012 (0.014)$

$+P \leq 0.05$, $**P \leq 0.005$, $***P \leq 0.001$
situational and social appraisals) with genetic variation on PEs differed between non-clinical and early-psychosis groups. As shown in Table 2, only the three-way interaction of situational stress, OXTR rs2254298, and group was significantly associated with PEs. This interaction indicated that situational stress was associated with increased PEs for participants with the A allele in the early-psychosis group. Simple slope analyses indicated that the A allele of the OXTR gene moderated the association between situational stress and PEs in the early-psychosis group but not in the non-clinical group (early-psychosis: 0.048, SE = 0.022, P = 0.031; non-clinical: –0.001, SE = 0.008, P = 0.927). In addition, analyses of the OXTR in the early-psychosis group showed that situational stress was associated with greater increases in PEs for AA and AG participants (AA: 0.255, SE = 0.063, P = 0.000; GA: 0.138, SE = 0.028, P < 0.001) as compared with those carrying the GG genotype (GG: 0.089, SE = 0.014, P < 0.001; see Fig. 2). As expected, dummy coding indicated that A allele carriers (AA and AG) experienced more PEs than GG subjects (AA vs. GG: 0.135, SE = 0.037, P = 0.000; GA vs. GG: 0.057, SE = 0.015, P = 0.000; AA vs. GA: 0.078, SE = 0.038, P = 0.038).

Finally, although some three-way interactions also seemed to emerge in COMT (rs4680), OXTR (rs53576), and BDNF (rs6265) models, these results did not reach significance after controlling for multiple testing.

Discussion

Findings

The present study extended previous GxE reports in psychosis by examining with ecological validity the interplay of genetic variants with both distal and proximal psychosocial environmental factors on the real-life expression of PEs. Both early-life and momentary (situational and social) stress were associated with increased levels of PEs, whereas none of the genetic variants studied were directly associated with PEs. GxE interactions of the risk haplotype of RGS4 and FKBP5 with distal, but not proximal, stress were associated with increased levels of PEs in the total sample. Moreover, when both groups were compared, results indicated that both factors were more strongly associated with PEs in the early-psychosis group as compared with the non-clinical group. In the early-psychosis group, the interactions of distal stress with the risk haplotype of RGS4 and FKBP5 with distal, but not proximal, stress were associated with the expression of PEs in the total sample. Moreover, when both groups were compared, results indicated that both factors were more strongly associated with PEs in the early-psychosis group as compared with the non-clinical group. In the early-psychosis group, the interactions of distal stress with the risk haplotype of RGS4 and FKBP5 were associated with increased levels of PEs, whereas the interaction of momentary situational stress with the A allele of OXTR (rs2254298) was associated with PEs.

The association of both distal and proximal environmental factors with PEs is consistent with a
Cristóbal-Narváez et al.

growing body of research showing that psychosocial stress—such as childhood trauma and momentary situational and social stress—is associated with schizotypy traits and subclinical and clinical expressions of the psychosis phenotype (7, 46–48). As expected, and consistent with previous studies (e.g., 9, 49), the association of both types of stressors with PEs was also greater in the early-psychosis group compared to the non-clinical group (even if the latter was oversampled for elevated scores on schizotypy to include a wide range of variability in terms of psychosis liability). Results thus seem to indicate that, although comparable mechanisms, such as stress sensitivity, operate across different levels of psychotic liability, and expression, there is also a differential impact of stressors in both groups reflecting individual differences in risk and resilience factors, some of which may pave the way toward psychotic outcomes. In addition, it may be that once PEs reach certain severity levels and need for care status the effect of stressors is of greater magnitude.

Our analysis of the moderating role of stress-regulation SNPs on environmental stress exposures showed that the interactions of the risk haplotypes of RGS4 and FKBP5 genes with distal, but not proximal, stress were associated with PEs in the total sample. Importantly, the group comparison indicated that such GxE findings only held for the early-psychosis group and, again, only for distal stress. This resonates with a recent study showing that, unlike momentary stress, the exposure of early-life stress may lead to long-lasting molecular mechanisms in relevant stress-response systems, shaping individual differential trajectories and resulting in a greater risk for the development of psychopathological outcomes (50). Prior studies have consistently demonstrated that exposure to childhood trauma increases the risk for several stress-related phenotypes for carriers of the minor alleles (C, A, T, T) of FKBP5 SNPs (rs3800373, rs9296158, rs1360870, and rs9470080; e.g., 51) or for the risk haplotype including these three or four alleles (e.g., 15, 52). Specifically, these risk alleles have been associated with decreased sensitivity of the glucocorticoid receptor (GR) to circulating cortisol, entailing a diminished negative feedback regulation of the HPA axis and hence, enduring responses to stress (51). Notably, a relevant finding consistent with the discrepancy of both stressors with FKBP5 variability is that specifically early-life stress (but not current cortisol levels or adult trauma exposure) in interaction with FKBP5 risk alleles induce epigenetic changes that result in individual differences in GR sensitivity, ultimately leading to the dysregulation of the stress response and an increased vulnerability for psychopathological phenotypes (15). These authors suggested that there may exist a sensitive development stage for such epigenetic changes altering the homeostasis of the HPA axis and causing subsequent abnormal responses to stress.

Similarly, the RGS4 risk haplotype moderated the association between distal, but not proximal, stress and PEs. Although, to the best of our knowledge, this is the first GxE study showing evidence of the interplay between psychosocial stress and RGS4 genetic variation on PEs, neurobiological research has indicated that the RGS4 gene, because of its function and biological properties, may be a relevant candidate gene for psychotic outcomes (25). RGS4 is highly expressed in important brain regions involved in the pathophysiology of schizophrenia (e.g., PFC) and, importantly, it also shows an increased responsiveness to environmental stimuli, can modulate the function of G-protein-coupled neurotransmitter receptors critically implicated in schizophrenia-related disorders (53, 54). Evidence from animal studies has also indicated that differential regulation of RGS4 expression may contribute to the dysregulation of the glucocorticoid-induced negative feedback regulation and thus, the prolongation of stress responses (55). In this context, our significant results of distal stress with the FKBP5 and RGS4 risk haplotypes suggest that after childhood trauma exposure, both variants may be involved in the HPA axis dysregulation associated with the risk for PEs across the extended psychosis phenotype. This is consistent with compelling evidence suggesting that the dysregulation of HPA axis may play a critical role in the expression of the positive dimension of psychotic phenomena (56, 57) due to the synergistic relation between glucocorticoid secretion and an elevated dopaminergic activity in mesolimbic regions (e.g., 58).

Conversely, the interaction of OXTR (rs2254298) A allele with momentary stress was associated with PEs in the early-psychosis group. Compelling evidence has shown that oxytocin administration has stress-buffering effects decreasing subjective stress experiences (28) and also diminishes amygdala activation in response to stressors (e.g., 59, 60). Notably, the two SNPs on OXTR (rs2254298, rs53576) have been associated with individual differences in intermediate mechanisms (e.g., affiliation, stress regulation, and empathy) underlying the risk for psychopathological phenotypes—especially those with social dysfunction features (e.g., 61). Although there is no prior evidence showing that, in particular, allelic variation (rs2254298) may moderate the association of
stress with PEs, several studies have linked this polymorphism with an increased risk for psychopathological outcomes (e.g., 62) and with alterations in important brain areas involved in stress reactivity and emotional responses, such as the hypothalamus and amygdala (e.g., 63). Interestingly, one study revealed that A carriers of rs225498 showed higher PANSS general symptom scores than GG individuals in a group of persons with schizophrenia, whereas no differences were found within a healthy control group (64). In light of these findings, it is attractive to speculate that early-psychosis individuals carrying at least one A allele of rs225498 may present a maladaptive regulation to negative environmental factors in the realm of daily life (e.g., stressful situations), increasing the susceptibility to psychopathology.

Conclusions and future directions

Overall, our results provide new insights into how the interplay of genetic variation within FKBP5, RGS4, and OXTR genes with distal or proximal environmental factors impacts the expression of PEs in early-psychosis compared to the non-clinical group. In addition, some interactions of environmental factors with BDNF and COMT variability also seemed to be associated with PEs; however, they did not reach statistical significance after controlling for multiple testing.

From a clinical view-point, the findings of this study add further support to the validity of the ESM approach in psychosis research by showing that early-psychosis individuals are able to meaningfully inform about their internal experiences, both in terms of symptoms and psychological appraisals of both context and interpersonal interactions. These issues are relevant for informing etiological models of symptom formation and may assist the development of ecological momentary interventions in psychiatry. In this regard, the ecological momentary interventions (EMIs) using experience sampling methodology recently devised in the field of psychosis constitute a promising and an innovative assessment and intervention approach, allowing to tailor-personalized interventions toward individual needs such as decreasing daily-life stress in the specific moment that are needed (65).

Strengths and limitations

The strengths of this study include the comparison of a non-clinical and early-psychosis sample, the use of ecologically valid measures of symptoms, and stress in real-life during multiple time points over a week enhancing the reliability of GxE research (66) and the estimation of two risk haplotypes increasing the power to detect genetic associations (67). Limitations of the study include its cross-sectional nature, which limits interpretations about the causal effects of GxE interactions. Similarly, causal inferences examining the effects of stress cannot be definitively drawn, given that predictor and criterion ESM variables were measured concurrently. It should also be noted that although the non-clinical sample was oversampled for schizotypy to insure adequate variance in psychosis liability, it does not constitute a homogenously high schizotypy sample. That is, the sample is composed of high, medium, and low schizotypy scorers, and the high scores refer to both the positive and negative schizotypy dimensions (which may involve different etiological mechanisms). Therefore, the sample composition precludes us from drawing precise conclusions of the role of schizotypy in the GxE interactions examined in the present study. Future research may focus on the examination of these GxE interactions in high and low schizotypy groups, analyzing the positive and negative dimensions independently.

Acknowledgements

This work was supported by the Spanish Ministerio de Economía y Competitividad (Plan Nacional de I+D PSI2014-54009-R; PSI2011-30221-C02-02 and C02-02; Red de Excelencia PROMOSAM PSI2014-56303-REDT), Fundació La Marató de TV3 (091110), and Generalitat de Catalunya (Suport als Grups de Recerca 2014SGR1070 and 2014SGR1636). NBV is supported by the Institució Catalana de Recerca i Estudis Avançats (ICREA), ICREA Academia Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. van Os J, Linscott RJ, Myn-Germeys I, Delespaul P, Krabbendam L. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. Psychol Med 2009;39:179–195.
2. Kwapił TR, Barrantes-Vidal N. Schizotypy: looking back and moving forward. Schizophr Bull 2015;41(Suppl 2): S366–S373.
3. McGorry PD, Nelson B, Goldstone S, Yung A. Clinical Staging: a heuristic and practical strategy for new research and better health and social outcomes for psychotic and related mood disorders. Can J Psychiatry 2010;55:486–497.
4. Barrantes-Vidal N, Grant P, Kwapił TR. The role of schizotypy in the study of the etiology of schizophrenia spectrum disorders. Schizophr Bull 2015;41(Suppl 2): S408–S416.
5. Reininghaus U, Kempton MJ, Valkmaglia L et al. Stress sensitivity, aberrant salience, and threat anticipation in early

Gene–environment interaction in early-psychosis
psychosis: an experience sampling study. Schizophr Bull 2016;42:712–722.
6. Varese F, Smeets F, Drukker M et al. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. Schizophr Bull 2012;38:661–671.
7. Barrantes-Vidal N, Chen C, Myin-Germeys I, Kwapi TR. Psychometric schizotypy predicts psychotic-like, paranoid, and negative symptoms in daily-life. J Abnorm Psychol 2013;122:1077–1087.
8. Velikova T, Fisher HL, Mason O, Johnson S. Childhood trauma and schizotypy: a systematic literature review. Psychol Med 2015;45:947–963.
9. Holtzman CW, Shapiro D, Troughton HD, Walker EF. Stress and the prodromal phase of psychosis. Curr Pharm Des 2012;18:527–533.
10. Uhé R. Gene-environment interactions in severe mental illness. Front Psychiatry 2014;5:48.
11. Holtzman CW, Troughton HD, Goulding SM et al. Stress and neurodevelopmental processes in the emergence of psychosis. Neuroscience 2013;249:172–191.
12. Alemay S, Arias B, Aguilera M et al. Childhood abuse, the BDNF-Val66Met polymorphism and adult psychotic-like experiences. Br J Psychiatry 2011;199:38–42.
13. de Castro-Catala M, van Niehop M, Barrantes-Vidal N et al. Childhood trauma, BDNF Val66Meth and subclinical psychotic experiences. Attempt at replication in two independent samples. J Psychiatr Res 2016;83:121–129.
14. Simons CJ, Wichers M, Derom C et al. Subtle gene-environment interactions driving paranoia in daily-life. Genes Brain Behav 2009;8:5–12.
15. Klei NL, Monda D, Ababy C et al. Allele-specific FKB5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci 2013;16:33–41.
16. Collip D, Myin-Germeys I, Wichers M et al. FKB5 as a possible moderator of the psychosis-inducing effects of childhood trauma. Br J Psychiatry 2013;202:261–268.
17. Aina Mah D, Borge S, di Forti M et al. Role of environmental confounding in the association between FKB5 and first-episode psychosis. Front Psychiatry 2014;5:48.
18. Alemay S, Moya J, Ibáñez MI et al. Childhood trauma and the rs1360780 SNP of FKB5 gene in psychosis: a replication in two general population samples. Psychiat Med 2016;46:221–223.
19. Cristóbal-Narváez P, Sheinbaum T, Rosa A et al. The interaction between childhood bullying and the FKB5 gene on psychotic-like experiences and stress reactivity in real life. PLoS ONE 2016;11:e0158809.
20. Csíkszentmihalyi M, Larson R. Validity and reliability of the experience sampling method. In: Devries MW, editor. The experience of psychopathology: investigating mental disorders in their natural settings. New York: Cambridge University Press; 1992: pp 34–57.
21. Henriksen JM, Schmidt JA, Csíkszentmihalyi M. Experience sampling method: measuring the quality of everyday life. Thousand Oaks: Sage; 2007.
22. van Winkel R, Heeselt C, Rosa A et al. Evidence that the COMT (Val158Met) polymorphism moderates sensitivity to stress in psychosis: an experience-sampling study. Am J Med Genet B Neuropsychiatr Genet 2008;147:10–17.
23. Collip D, van Winkel R, Peerbooms O et al. COMT Val158Met-stress interaction in psychosis: role of background psychosis risk. CNS Neurosci Ther 2011;17:612–619.
24. Peerbooms O, Rutten BP, Collip D et al. Evidence that interactive effects of COMT and MTHFR moderate psychotic response to environmental stress. Acta Psychiatr Scand 2012;125:247–256.
25. Buckholtz JW, Meyer-Lindenberg A, Honka RA et al. Allelic variation in RGS4 impacts functional and structural connectivity in the human brain. J Neurosci 2007;27:1584–1593.
26. Steffans NC, Trikalinos TA, Avramidopoulou D et al. Association of RGS4 variants with schizotypy and cognitive endophenotypes at the population level. Behav Brain Funct 2008;4:46.
27. de Castro-Catala M, Cristóbal-Narváez P, Kwapi TR et al. Association between RGS4 variants and psychotic-like experiences in nonclinical individuals. Eur Arch Psychiatry Clin Neurosci 2016;267:19–24.
28. Kümsta R, Heinrichs M. Oxytocin, stress and social behavior: neurogenetics of the human oxytocin system. Curr Opin Neurobiol 2013;23:11–16.
29. Shamay-Tsoory SG, Abu-Akel A. The social salience hypothesis of oxytocin. Biol Psychiatry 2016;79:194–202.
30. Chapman LJ, Chapman JP, Raulin ML. Scales for physical and social anhedonia. J Abnorm Psychol 1976;85:374–382.
31. Chapman LJ, Chapman JP, Raulin ML. Body image aberration in schizophrenia. J Abnorm Psychol 1978;87:399–407.
32. Eckblad M, Chapman LJ. Magical ideation as an indicator of schizotypy. J Consult Clin Psychol 1983;51:215–225.
33. Eckblad ML, Chapman LJ, Chapman JP, Miehlove M. The Revised Social Anhedonia Scale. University of Wisconsin; Madison, 1982. Unpublished test.
34. Raine A. The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. Schizophr Bull 1991;17:555–564.
35. Steffans NC, Hanssen M, Smeets NK et al. Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med 2002;32:347–358.
36. Domínguez-Martínez T, vanier E, Massanet MA, Torices I, Jané M, Barrantes-Vidal N. The need-adapted integrated treatment in Sant Pere Claver-Early Psychosis Program (SPC-EPP) in Barcelona, Spain. Salud Ment 2011;34:517–524.
37. Yung AR, Yuen HP, McGorry PD et al. Mapping the onset of psychosis—the Comprehensive Assessment of At Risk Mental States (CAARMS). Aust NZ J Psychiatry 2005;39:964–971.
38. Schultze-Lutter F, Addington J, Ruhmann S, Klosterkötter J. Schizophrenia proneness instrument—adult version (SPI-A). Rome: Giovanni Fioriti; 2007.
39. First MB, Spitzer RL, Gibbon M, Williams JB. Structured clinical interview for DSM-IV Axis I Disorders-Patient ed. (SCID-I/P, Version 2.0). New York: Biometrics Research Department, 1995.
40. Bernstein DP, Fink L. Childhood Trauma Questionnaire: a retrospective self-report manual. San Antonio, TX: The Psychological Corporation; 1998.
41. Barrett JC, Fry B, Maller J, Daly MJ. Haplovie: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265.
42. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2013;73:1162–1169.
43. Zannas AS, Binder EB. Gene-environment interactions at the FKB5 locus: sensitive periods, mechanisms and pleiotropy. Genes Brain Behav 2014;13:25–37.
44. IBM CORP. IBM SPSS Statistics, version 19.0. IBM Corp: Armonk, 2010.
45. STATACORP. Stata Statistical Software, release 12. StataCorp LP; College Station, 2011.
Gene–environment interaction in early psychosis

46. MYIN-GERMEYS I, van Os J, SCHWARTZ JF, STONE AA, DELSPAAN PA. Emotional reactivity to daily-life stress in psychosis. Arch Gen Psychiatry 2001;58:1137–1144.

47. CRISÍTÓBAL-NARVÁEZ P, SHENBAUM T, BALLESPÍ S et al. Impact of adverse childhood experiences on psychotic-like symptoms and stress reactivity in daily-life in nonclinical young adults. PLoS ONE 2016;11:e0153557.

48. REININGHAUS U, GAYER-ANDERSON C, VALMACchia I, et al. Psychological processes underlying the association between childhood trauma and psychosis in daily-life: an experience sampling study. Psychol Med 2016;42:1–15.

49. TROTMAN HD, HOLTZMAN CW, WALKER EF et al. Stress exposure and sensitivity in the clinical high-risk syndrome: initial findings from the North American Prodrome Longitudinal Study (NAPLS). Schizophrenia Res 2014;160:104–109.

50. KLENGEL T, BINDER EB, FKBP5 allele-specific epigenetic modification in gene by environment interaction. Neuropsychopharmacology 2015;40:244–246.

51. BINDER EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology 2009;34(Suppl 1):S186–S195.

52. BINDER EB, Salyakina D, LICHTNER P et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet 2004;36:1319–1325.

53. CHOWDHARI KV, BANSE M, WOOD J et al. Linkage disequilibrium patterns and functional analysis of RGS4 polymorphisms in relation to schizophrenia. Schizophrenia Bull 2008;34:118–126.

54. LEVITT P, EHRIT P, MIRNICS K, NIMMOGONKAR VL, LEWIS DA. Making the case for a candidate vulnerability gene in schizophrenia: convergent evidence for regulator of G-protein signaling 4 (RGS4). Biol Psychiatry 2006;60:534–537.

55. Ni YG, GOLDS SJ, IREDALE PA, TERRILLGER RZ, DAMAN RS, NESTLER EJ. Region-specific regulation of RGS4 (Regulator of G-protein-signaling protein type 4) in brain by stress and glucocorticoids: in vivo and in vitro studies. J Neurosci 1999;19:3674–3680.

56. KAPUR S. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. Am J Psychiatry 2003;160:13–23.

57. READ J, van OS J, MORRISON AP, ROSS CA. Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. Acta Psychiatr Scand 2005;112:330–350.

58. van WINKEL R, STEFANIS NC, MYIN-GERMEYS I. Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull 2008;34:1095–1105.

59. DOMES G, HEBRICH M, GLAŚCHER J, BÜCHEL C, BRAUS DF, HEBERTZ SC. Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol Psychiatry 2006;62:1187–1190.

60. PETROVIC P, KALISCH R, SINGER T, DOLAN RJ. Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. J Neurosci 2008;28:6607–6615.

61. FELDMAN R, MONAKHOV M, PRATT M, ERSTEIN RP. Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. Biol Psychiatry 2016;79:174–184.

62. THOMPSON RJ, PARKER KJ, HALLMAYER JF, WAUGH CE, GÖTTLI H. Oxytocin receptor gene polymorphism (rs2254298) interacts with familial risk for psychopathology to predict symptoms of depression and anxiety in adolescent girls. Psychoneuroendocrinology 2011;36:144–147.

63. TOST H, KOLACHANA B, HAKIM S et al. A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic limbic structure and function. Proc Natl Acad Sci USA 2010;107:13936–13941.

64. MONTAG C, BROCKMANN EM, LEHMANN A, MÜLLER DJ, RUESCU D, GALLINAT J. Association between oxytocin receptor gene polymorphisms and self-rated ‘empathic concern’ in schizophrenia. PLoS ONE 2012;7:e51882.

65. MYIN-GERMEYS I, KLEPPEL A, STEINHART R, REININGHAUS U. Ecological momentary interventions in psychiatry. Curr Opin Psychiatry 2016;29:258–263.

66. MYIN-GERMEYS I, OORESCHOT M, COLLIP D, LATASTER J, DELSPAAN P, van OS J. Experience sampling research in psychopathology: opening the black box of daily-life. Psychol Med 2009;39:1533–1547.

67. CRAWFORD DC, NICKERSON DA. Definition and clinical importance of haplotypes. Annu Rev Med 2005;56:220–303.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Demographic characteristics, ESM and genetic variables across the extended psychosis phenotype.

Table S2. Details on the genetic polymorphisms included in the present study.

Table S3. Principal component analysis (PCA) of ESM PEs measure.