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Research paper

Oxytocin receptor and G-protein polymorphisms in patients with depression and separation anxiety

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ABSTRACT

Background: The impact of combined variants of Oxytocin Receptor (OXTR) and G protein β3 subunit genes was investigated in relation to retrospective reports of childhood as well as contemporary adult separation anxiety (SA), based on evidence of a β/γ dimer-mediated signaling for OXTR.

Methods: A case-control association study (225 healthy adults and 188 outpatients with depression) was performed to establish Risk-Combined Genotype (RCG) of the studied variants (OXTR rs53576 and the functional Gβ3 subunit rs5443). Current SA was evaluated by the ASA-27 and retrospective childhood symptoms by the SASI. GG genotype of OXTR rs53576 combined with T-carrier genotype of Gβ3 rs5443 represented the RCG.

Results: Compared to non-RCG, those with RCG had significantly higher levels of childhood and adult SA. The RCG was significantly associated with childhood SA threshold score (OR=2.85, 90%CI: 1.08–7.50). Childhood SA was, in turn, strongly associated with a threshold SA score in adulthood (OR=15.58; 95% CI: 4.62–52.59).

Limitations: Although the overall sample size is sizable, comparisons among subgroups with specific combination of alleles are based on relatively small numbers.

Conclusions:: Our study indicates that variations in OXTR and Gβ3 genes are specifically associated with presence and severity of SA in childhood and adulthood, but not with depression or anxiety in general. Because there is increasing interest in oxytocin in social behavior, the gene-SA associations identified have potential translational and clinical relevance.

1. Introduction

Depressive disorders are thought to result from the interplay of multiple genes interacting with environmental factors (Swaab et al., 2005; Grippo et al., 2007). There is substantial literature suggesting the involvement of the oxytocin (OXT) in depression based on evaluation.

of its levels in plasmatic/cerebrospinal fluid and of OXT transcripts in post mortem tissues of depressed patients (Scantamburlo et al., 2007; Wang et al., 2008). From this perspective, the traditional view of OXT as an endocrine hormone acting on peripheral organs (i.e., to induce labor and milk ejection) has been revised (Gimpl and Fahrenholz, 2001). Oxytocin is now considered to be a neurotransmitter or neuromodulator with central actions in the limbic system, particularly the amygdala, a key structure involved in mood disorders (Kirsch, 2015). Previous studies have documented modifications of neural activity induced by OXT in limbic regions of depressed patients (Pincus et al., 2010) and in animal models of depression (Slattery and Neumann, 2010).

In numerous studies, depression has been found to be strongly associated with separation anxiety, a condition characterized by apprehension over rejection, abandonment and high sensitivity to real or perceived threats to relationships (Carnelley et al., 1994; Murphy and Bates, 1997; Mickelson et al., 1997; Bifulco et al., 2002; Shaver et al., 2005; Conradi and De Jong, 2008). Separation from close

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attachment figures induces anxiety, which is normal and adaptive in early childhood. If this emotional state persists in its more severe form into adulthood, the individual feels unable to function alone in the absence of a reference person with a negative impact on depressive symptomatology, quality of life and outcome of treatment for mood disorders (Pini et al., 2014; Milrod et al., 2014; Marnane and Silove, 2013). The estimated lifetime prevalence of adult separation anxiety disorder occurring alone is 4.8%, but rate of comorbidity with depressive disorders reaches about 30% (Shear et al., 2006; Pini et al., 2014; Silove et al., 2015). Most studies, which attempted to identify the biological foundations of separation anxiety, pointed out the important role of OXT. Milrod and colleagues (Milrod et al., 2014) reappraised all available data on OXT in this context, concluding that altered plasma OXT levels are associated with greater anxiety and relationship dissatisfaction in persons with separation anxiety disorder. As might be expected given the strong evolutionary conservation of the small nonapeptide OXT, mutation analysis of the corresponding gene, as a whole, has shown no consistent disturbance in separation anxiety disorder in adulthood (Costa et al., 2009a). A positive genetic association with separation anxiety disorder in adulthood has been found however, for a single nucleotide polymorphism (SNP; rs53576) of the OXT Receptor (OXTR) gene (Costa et al., 2009b). Specifically, the GG genotype of this SNP has been linked to high levels of separation anxiety and insecure attachment in patients with major depression (Costa et al., 2009b). Other authors have found that an interaction between exposure to high levels of early adversity with another OXTR SNP (rs2254298) is associated with the level of separation anxiety (SA) symptoms in adolescents (Thompson et al., 2011). Although the precise genetic function of OXTR rs53576 remains to be established, a study suggests it may determine deficits in socio-behavioral domains such as separation sensitivity, attachment and positive affect (Tost et al., 2010). Interest in the potential psychiatric relevance of this genetic variant was furthered by studies indicating that rs53576A is over-transmitted in some families with offspring exhibiting autistic spectrum disorders and may represent a central component of haplotypes relevant to high-functioning autism (Wermter et al., 2009).

The SNP rs53576 is located within the third intron of the OXTR gene which appears to be instrumental in the epigenetic regulation of OXTR expression (Mizumoto et al., 1997). The OXTR is a prototypical G protein-coupled receptor (GPCR) known to couple to heterotrimeric Gq/11 protein. Contrary to the classical perspective on GPCR signaling, the β/γ subunits are the major mediators of OXT-evoked activation of intracellular signaling, including both to peripheral cells and neurons (Zhong et al., 2011). In magnocellular neurons, the release of βγ subunits induced by OXTR activation has been shown to play a prominent role in generating burst firing patterns, indicating that the dimer is crucial for pulsatile neuropeptide secretion (Wang and Hatton, 2007).

In our efforts to extend knowledge about the role of the OXT pathway in separation anxiety we explore the role of a functional SNP (rs5443) of β3 subunit of G protein gene in addition to that of OXTR rs53576) (Rosskopf et al., 2003; Ruiz-Velasco et al., 2003), given that the former may be involved in a intracellular signaling pathway activated by the stimulation of OXTR. Our focus is also influenced by the knowledge that the β3 subunit isoform of G protein is ubiquitously expressed in the brain, including in oxytoninergic regions (Liang et al., 1997; Zhong et al., 2003; Wang et al., 2007).

The T allele of rs5443 causes an alternative splicing of exon 9 resulting in an in-frame deletion of 123-bp. Gβ3 splice variant (termed Gβ3s) is 41 amino acids shorter than the wild-type Gβ3. Although this issue has been not definitively clarified, there is evidence of an inability of Gβ3 s to activate a variety of intracellular effectors (Siffert et al., 1998).

As described above, data from literature show a strong association between oxytocin pathways and depression. Parallel, converging evidences pointed out the essential role of OXT in the biological foundations of attachment behaviours of which separation anxiety represents one of most important clinical correlates among adult individuals (Milrod et al., 2016). Within this framework, in a previous study, we found a significant association between OXTR polymorphisms and several attachment dimensions among depressive patients (Costa et al., 2009b). All these data, prompted us to explore, in patients with depression, whether combined genotypes of OXTR rs53576 and Gβ3 rs5443 were associated with either childhood or adult separation anxiety and their relationship with depressive symptoms.

2. Method

2.1. Subjects selection

The study sample comprised 413 participants, of which 225 were healthy individuals. The remaining sample included an overall group of 188 consecutive adult psychiatric outpatients with Axis I mood disorders as a principal diagnosis referred to the outpatients' clinic of the Department of Psychiatry at the University of Pisa. Patients with psychotic disorders, substance abuse and serious medical conditions were excluded from the analyses. The study sample represented an extension of a sample used in a previous our work (Costa et al., 2009b). All recruited patients and controls were Caucasians on the basis of a retrospective analysis of their genealogy departing from three generations behind, according to previous studies (Fuku et al., 2015; Tannorella et al., 2016). Healthy individuals were recruited among university personnel and were assessed by SCID-I-NP (First et al., 2002a) and additional medical screening. Volunteers with a current or past history of major psychiatric disorders were excluded. The University of Pisa Ethical Committee approved the study design. All subjects provided written informed consent prior to participation after being informed of the nature of study procedures.

2.2. Psychometric evaluation

All patients were assessed with the SCID-I (First et al., 2002a, 2002b) to establish a DSM-IV Axis-I diagnosis and patterns of psychiatric comorbidity and were specifically screened for lifetime separation anxiety disorder. Patients were evaluated by the Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960) to assess the severity of depression. Anxiety was assessed by the 'Anxiety' Factor score of the HAM-D (McClintock et al., 2011).

2.3. Assessment of separation anxiety

2.3.1. Adult separation anxiety – checklist (ASA-27)

The ASA-27 is a self-report questionnaire containing 27 items assessing relevant symptoms in adulthood (Manicavasagar et al., 2003). Participants respond to items on a four-point Likert scale, ranging from "This has never happened" to "This happens very often". Item scores (0–3) are added to yield a total score ranging from 0 to 81. The measure has shown a high level of internal consistency (Cronbach's alpha=0.89), and test–retest reliability ($r = 0.86$, $p < 0.001$) (Manicavasagar et al., 1997; Silove and Marnane, 2013). A total score of 22 or higher provides a threshold for adult separation anxiety that has been shown to correspond closely to a diagnosis of separation anxiety disorder based on a structured clinical interview (Manicavasagar et al., 2003).

2.3.2. Separation Anxiety Symptom Inventory (SASI)

The SASI is a 15-item self-report measure assessing separation anxiety symptoms retrospectively, based on experiences prior to 18 years of age (Silove et al., 1993). Items are scored from 0 to 3 on a frequency scale. The SASI has been shown to have sound internal (Cronbach's alpha=0.88) and test-retest reliability over 24 months (intraclass correlation coefficient=0.89). In the development of the